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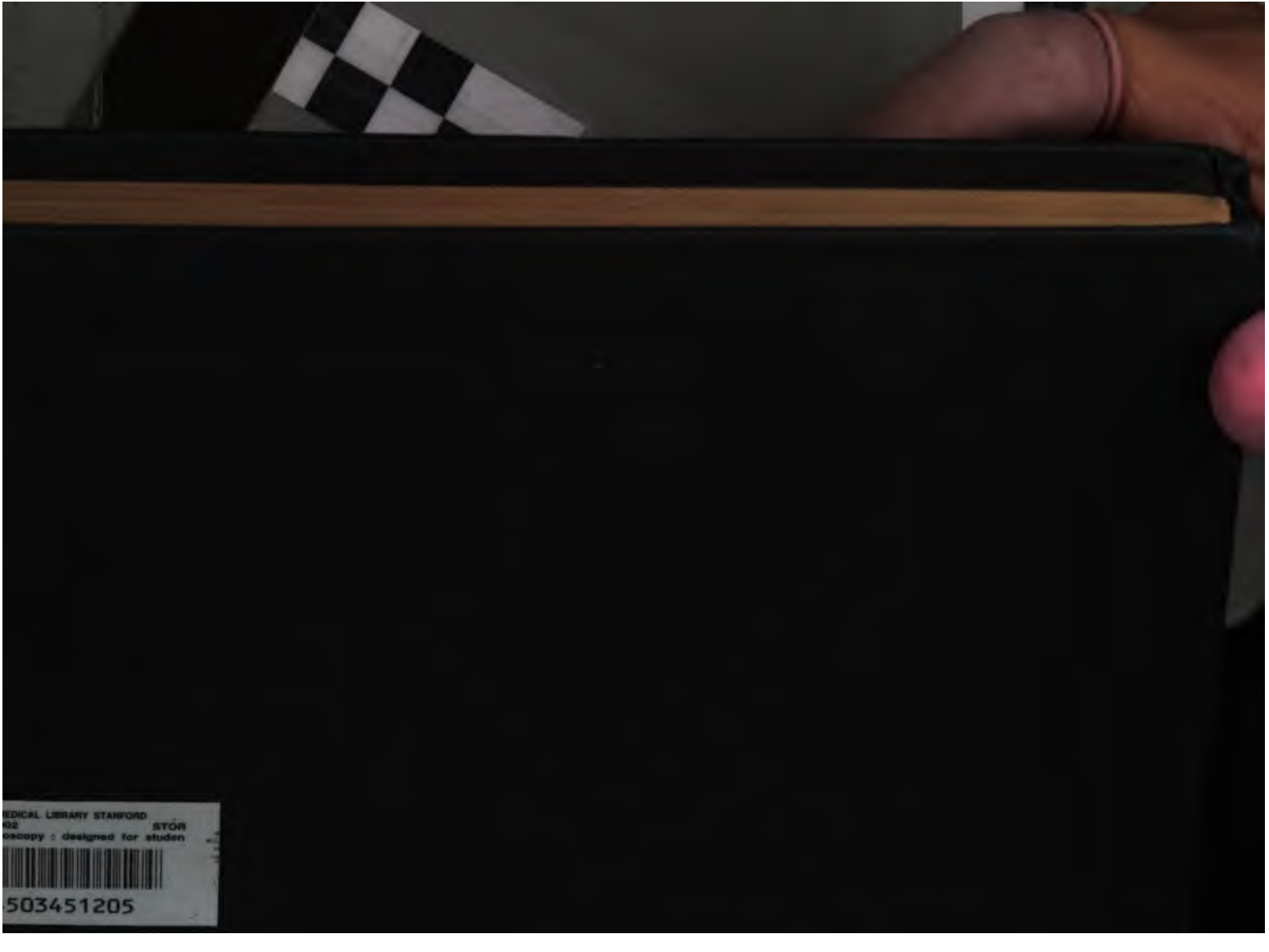
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MEDICAL MICROSCOPY.

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MEDICAL MICROSCOPY

DESIGNED FOR STUDENTS IN LABORATORY WORK AND FOR PRACTITIONERS

BY

T. E. OERTEL, M.D.

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With 131 Illustrations, some of which are Colored

PHILADELPHIA

P. BLAKISTON'S SON & CO.

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PREFACE.

In this day of multiplicity of medical books none should venture to afflict the medical public with a volume which is not called for by some legitimate voice. Believing this, I have still brought forth the following work.

Microscopy is a new and a growing science. Those of the profession who were graduated more than a decade ago for the most part received scanty instruction in laboratory work, and since that time much has been added to the technique of medical microscopy. There are many who are unable to avail themselves of a post-graduate laboratory course who desire to do such microscopic investigation as will be helpful to them in their daily practice. The more complete works on microscopy offer to such an one a bewildering mass of material from which he is unable to cull that which will be of service to himself.

It is to the beginner in microscopy, and particularly to him who must work without the advantage of the personal guidance of a teacher, that the book may prove of value.

It will be noticed that usually only one method is given whereby to reach a certain result. This is the method which has proved the best for routine work and which is at the same time the most simple and the least liable to error.

Claim of originality can be made only in the matter of presentation of the subject. The work is necessarily a

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compilation from authorities upon the questions of which it treats.

I am particularly indebted to Mr. Wm. Bausch for the use of cuts from his valuable little book, "Manipulation of the Microscope," and much of the material in the first section has been gleaned from the same source.

Nearly all the text-books upon clinical microscopy, bacteriology, and pathology have been consulted and credit given in the text wherever direct quotation has been made.

I wish to express my thanks to my friend, Mr. Wm. R. McKinley, to my assistants, Drs. Kellogg and Fargo, and to my wife for aid in proofreading and indexing the work, and to Messrs. P. Blakiston's Son & Co. for their uniform courtesy and their skilful product of the bookmaker's art.

THE AUTHOR.

AUGUSTA, GEORGIA, *November* 1, 1902.

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MEDICAL MICROSCOPY.

THE MICROSCOPE.

The word microscope is derived from the Greek μικρός, "small," and σκοπος, "a spy."

Microscopes are of two forms—simple and compound. A simple microscope consists of a single lens or a combination of lenses in close contact and united by a cement, and with this we view an object directly and see an image of it in an upright position.

A compound microscope is composed of two lenses or systems of lenses, the lower, called the objective, forming an image which is again magnified by the upper lens or ocular.

In the compound microscope the rays of light forming the image cross in their passage between the objective and ocular, so that we see a *reversed* picture of the object, and it appears upside down.

Lenses.—When a ray of light passes from one transparent medium to another of greater or less density, it is deflected from its original course; this action is called *refraction*, and upon this physical quality is based the science of optics.

If we view an object through a glass prism, it will be seen to be in a plane different from its real one, and the amount of deviation thus caused is dependent upon the

density of the glass from which the prism is formed and also upon the angle of the sides of the prism.

A ray of light passing through a prism is refracted when it enters the surface and again when it emerges (Fig. 1), and is *always bent toward the base* of the prism.

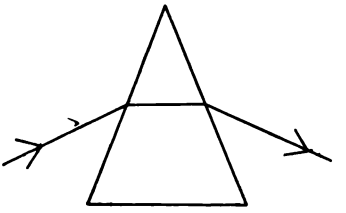

FIG. 1.

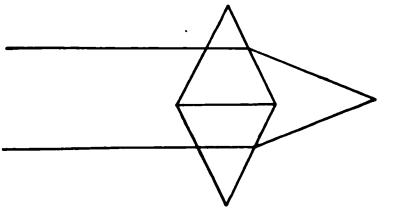

FIG. 2.

If we place two prisms with their bases together, the effect upon a ray of light passing through each would be as shown in Fig. 2; and if the position of the prism is reversed, the rays of light would pass as shown in Fig. 3.

Lenses are of two principal forms, and each of these is

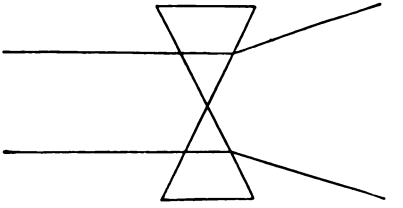

FIG. 3.

in reality only a combination of prisms with curved surfaces (Fig. 4). If the bases of the prisms are placed together, we have resulting a convex lens which will bring together or *converge* rays of light passing through it, while if the bases are placed outward, there results a *concave*

lens which separates or *diverges* rays of light which pass through it.

In Fig. 5 are shown the various forms of convex and concave lenses—double convex, plano-convex, convex-meniscus, and double-concave, plano-concave, concave-meniscus.

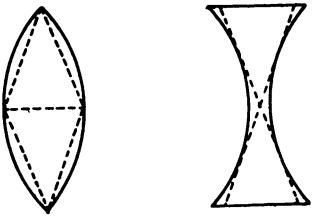

FIG. 4.

We have seen that parallel rays *a*, *c'* passing through a convex lens are converged (Fig. 6). The point at which these rays meet and cross is called the *focal point*, or *principal focus*, and the distance from the center of the lens *b* to *c* is the focal distance.

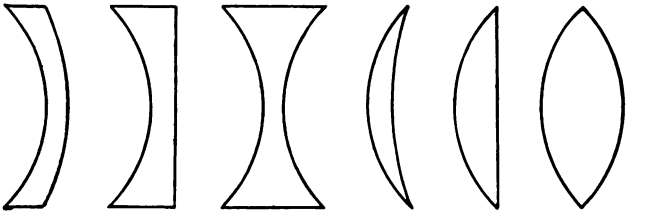

FIG. 5.

The line *d c* represents the principal axis of the lens and the line *a c'* its radius. Upon the radius or amount of curvature of the surface of a lens depends its power of convergence, so that as the radius becomes longer the magnifying power of the lens is decreased.

Parallel rays impinging upon a concave lens are diverged, and such lenses are called dispersing lenses (Fig. 7).

As the rays are never united, we have no actual image, but only an apparent or virtual image, which lies upon the

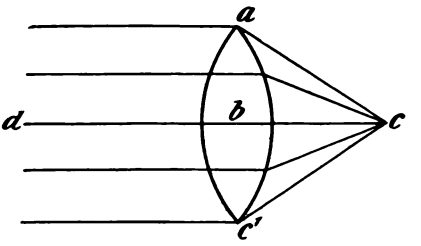

FIG. 6.

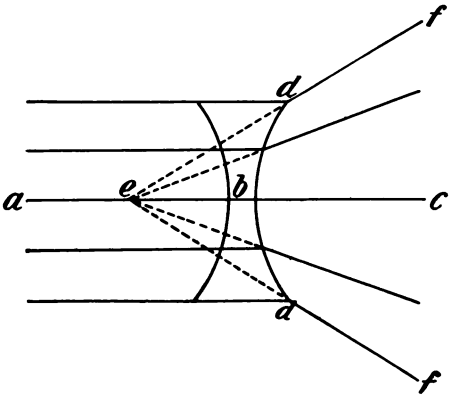

FIG. 7.

same side as the object from which the rays come. The point where this image is formed is called the virtual focus.

We have also a virtual focus in a convex lens formed upon the same side of the lens as is the object viewed by the projection of the rays.

The rays coming from the object are converged and impinge upon the eye (*e*, Fig. 8); and if the lines between *c* and *e* be sufficiently elongated, we find that they come together and form a virtual magnified image at *a b*.

Spherical Aberration.—It has not been mentioned that these rays passing through the margin of a double convex

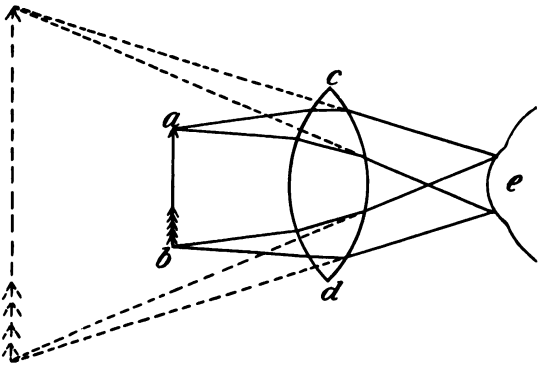

FIG. 8.

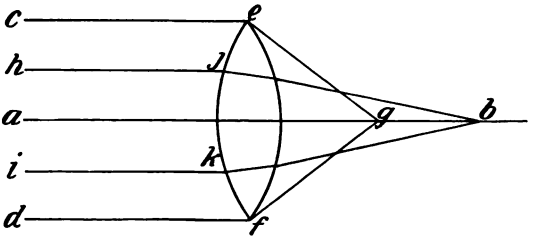

FIG. 9.

lens are refracted more strongly than those passing through its center. Such is the case, however, and we get in consequence a blurred image when we view an object through the periphery of the lens, while in the center it is clear and well defined. This quality is called spherical aberration.

In Fig. 9 we have a graphic illustration of this effect, and here it will be seen that the marginal rays *e c* being strongly refracted at *e*, meet at *g*, while the central rays are brought to a focus at *f*. This defect can be partially remedied by employing lenses of different form, and is least in a lens in which the radii of the two surfaces differ as 1:6 and the surface of the shorter radius is placed toward the object.

The most efficient method of decreasing spherical aberration is by the use of a diaphragm or stop, before or behind the lens, which consists of a disc having a circular opening and which cuts off the confusing marginal or *aberrant* rays and allows only the central rays to proceed and form the image.

Chromatic Aberration. — A beam of light passing through a prism is broken up or dispersed into its primary constituents, so that we have formed the colors of the spectrum—violet, indigo, blue, green, yellow, orange, and red. As we have seen, every lens, of whatever form, is virtually a prism, and therefore we have in all lenses a greater or less degree of chromatic aberration manifested by color fringes about the periphery of the field and of objects therein.

The violet end of the spectrum is more refrangible, and will be first brought to a focus, within the principal focus of the lens.

In Fig. 10 we see that the ray *a b*, impinging upon the surface of the prism, divides, and that upon its emergence the violet ray *v v'* is at some distance from the red ray *r r'*.

This effect may be entirely neutralized by placing in the path of these rays a second prism of the same form and density as the first, with the base directed upward; the rays being now brought together, emerge from the second prism as white light.

But the ray *c d* now takes the same direction as the ray *a b*, and as we must have converging rays in order to obtain a magnified image, it is not possible to correct chromatic aberration in this manner.

It may in a measure be corrected by combining two

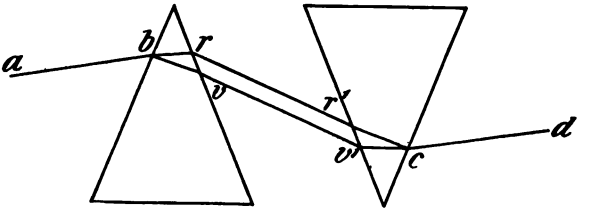

FIG. 10.

lenses of different density, and consequently of different dispersive power, the one partially neutralizing the effect of the other and producing what is known as an achromatic lens.

Such a form is shown in Fig. 11, the convex lens being of crown glass while the concave is of flint glass. This is the simplest form of *corrected* lenses and meets all ordinary requirements.

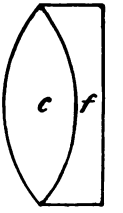

FIG. 11.

An **aplanatic** lens is one in which the chromatic and spherical aberrations are corrected.

It must be remembered that opticians have not yet succeeded in producing a lens of perfect correction, but the higher grades approach perfection in this respect.

In the compound microscope the objective is the lower lens or system of lenses and forms the first image, which is afterward again magnified by the eyepiece.

Objectives are of two kinds—dry and immersion. In the dry, air is the medium intervening between the front

of the bottom lens of the objective and the upper surface of the cover-glass. Optical difficulties increase with magnifying power, and therefore the higher power lenses are constructed with a view to filling up the space between the front lens of the objective and the cover-glass with a medium of the same refracting power as the crown glass of which the lens is constructed, for purposes to be explained. Water and glycerin were formerly used, but it has been found that thickened cedar oil best meets the requirements, and such a fluid is known as homogeneous immersion fluid, or is, for the sake of brevity, generally called simply *oil.*

It is of vital importance to remember that the highest efficiency can be obtained from a lens only when the oil used is of the same refractive and dispersive power as the hemisphere of the objective, and for this reason the oil furnished by the maker of the particular lens in question should alone be used.

Naming of Objectives.

Unfortunately until recently each maker has been a law unto himself in naming objectives, many having been designated by arbitrary numbers and letters. In this country there has long been a standard, and the European firms are gradually adopting it. According to this standard, objectives have marked upon them a focal length which indicates their magnifying power. The value of an objective is arrived at by comparing it with a *simple lens* of equal magnifying power and marking the objective with the focal length of the simple lens.

If the combined lenses of an objective give the magnifying power equal to that of a simple lens of one inch focus, the objective is marked one inch, or if the image from the system of an objective equals in power that of a

simple lens having the focus of $\frac{1}{16}$, the objective is marked $\frac{1}{16}$.

As the magnifying power of a lens increases in ratio with a decrease in the focus, a 1 inch objective having an initial or real magnifying power of ten diameters, a $\frac{1}{10}$ inch objective will magnify ten times this or 100 diameters, a $\frac{1}{12}$ inch will magnify twelve times this or 120.

Powers.—Objectives range in power from 5 inch to $\frac{1}{50}$ inch. In ordinary work it is found inexpedient to use higher powers than $\frac{1}{16}$, and most workers prefer a $\frac{1}{12}$ inch, which gives sufficient magnification for bacteriologic and blood work.

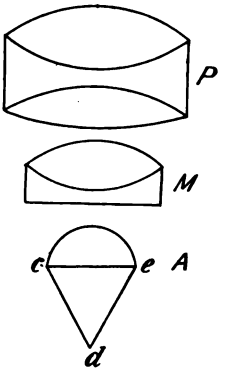

FIG. 12.

Angular Aperture.—In Fig. 12 the angle *C D E* represents the angular aperture of the objective. It is therefore the angle which is made by the most extreme rays which can enter the lens from the point of focus.

This is true only of objectives where all the rays entering the front lens are utilized in forming an image, and does not hold good in those objectives in which the marginal rays are not all of service, as would be the case if a diaphragm were employed behind the front lens.

Objectives of the same power may differ in angle, while those of unequal power may have the same angle. Definition is directly dependent upon angular aperture, for the reason that as light rays proceed from an object equally in every direction that lens which from a given surface will collect the most rays will show us the object more plainly.

This is well illustrated in Fig. 13 and Fig. 14. Here we

have two lenses of equal power and focus, *a*, *b*, *c* being the angle of aperture in each case. But the lens in Fig. 13 transmits more rays than that in Fig. 14, and will therefore produce the more distinct image of the two, though the picture will be no larger than that of Fig. 14.

Thus we find that lenses of the same angular aperture will produce an image of equal *distinctness*, though one be of much *lower power* than the other.

Numerical aperture is the ratio between the focal length of the objective and the diameter of the emergent pencil of light from the back of the objective.

The efficiency of an objective depends directly upon its numerical aperture, as was first pointed out by Professor Abbe.

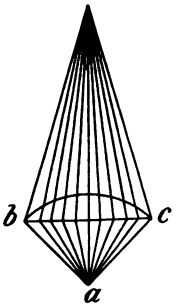

FIG. 13.

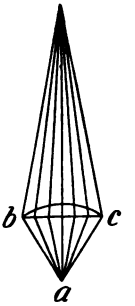

FIG. 14.

Upon objectives the term is indicated by the abbreviation N. A.

Resolving Power.—By this is meant the ability of an objective to bring to view minute details. If an objective is properly corrected for spherical aberration, its power of definition or resolving power is in direct ratio to its numerical aperture.

It goes without saying that this is the chief characteristic of a good objective, as our object is to see such structures as we wish to examine not only enlarged, but with the greatest possible distinctness.

By reference to the "Aperture Table" published by the Royal Microscopical Society we will find that an objective of twice the aperture of another will resolve or show twice the number of fine lines. One with an aperture of 0.05 will

resolve 4.821, and one with an aperture of 0.10 will resolve 9.641 lines to the inch.

It will be found necessary when viewing fine structural details to use not only a lens of sufficient angular aperture to define the details, but also to magnify them so that the eye will be able to distinguish them.

The number of ruled lines that the normal eye is capable of seeing unaided is about 200 to the inch. We must, therefore, use an objective which will have power enough to bring fine details up to at least this magnification, while at the same time its aperture is such as to enable us to see them distinctly, for we may have an image of much greater magnification with a lens of lower aperture which is indisinct or *unresolved.* We wish not to see an object as *large* as possible, but to see its details as *clearly* as possible.

The power of magnification may be increased in several ways; either by using an objective of higher power, by using an eye-piece of higher power, or by increasing the tube length. Suffice it to say that the first method is the preferable one, as by high eye-piecing we lose light and definition and magnify the defects of the initial image of the objective, and by increasing the tube length fall into the same and other difficulties.

Penetration.—By this we understand the power to look down into the structure of an object, as in a piece of tissue to see several cells each lying in different planes, at the same time without refocusing. This quality is in inverse ratio to power and angular aperture. With a $\frac{2}{3}$ inch objective we can see much deeper into a tissue than we can with a $\frac{1}{6}$ inch, and with lenses of the same power but of different angle we would find greater penetration in the one of the lower angle. Penetration is a quality which is of some importance to the worker in tissues as it enables

him to see the relations of cells without undue and continued focusing. One must not lose sight of the fact that after all resolution is more to be desired, and an objective of wide aperture should not be condemned because it is lacking in the quality of penetration.

Chromatic Aberration.—We have seen that a prism of any angle will break up a pencil of light into its primary colors, thus forming the spectrum, and also that a lens of any form is a modified prism and that the correction of an objective for color is accomplished by combining glasses of proper density.

It is not possible, however, entirely to reunite the light ray and produce white light. In the achromatic lenses only two colors, red and violet, are eliminated, the remaining rays forming green and purple, and this is known as the **secondary spectrum,** and we find it most apparent in objectives of high aperture and increased when high oculars are employed.

In testing an objective some form of diatome is generally used; and if when using such the light from the mirror is made to fall upon it obliquely, a fringe of color will be noticed around its edges, yellow-green upon the side opposite to that from which the light comes, and violet upon the same side as that from which the light comes. For ordinary work this has little bearing upon the efficiency of the lens. When central light is employed, color fringes are obviated to a large extent, and in stained tissue sections we seldom see them. If the objective is *under*-corrected we will observe that with central light we obtain about our test object a blue color if the focus is slightly above the object, an orange color if we focus the lens on a point just beneath it. If the objective is *over*-corrected the reverse effect will be obtained. Oblique light will bring colors more clearly into view.

The **apochromatic** objectives first made by Zeiss are corrected for three spectral colors and are more perfectly corrected for spherical aberration than the achromatic objectives. It is also possible to obtain in these objectives a much higher magnification than in the achromatics of the same power, and consequently they are superior in resolving power. The uncorrected error which remains in these lenses is partly overcome by using an eye-piece correspondingly overcorrected, and these are called *compensating oculars.*

It is a matter of regret that in the Zeiss apochromats one of the systems contains a lens composed of fluorite which frequently, in our climate, degenerates and renders the objective valueless.

The Bausch & Lomb Optical Company now list a series of apochromats after the computation of Professor Hastings, of Yale, which they claim are equal in performance to any and are not subject to the above grave fault.

For photomicrography apochromats are invaluable on account of their higher resolving power, flatness of field, and the fact that in them the visual and chemical rays are coincident; so that no allowance in focusing must be made.

Flatness of Field.

If we focus an objective upon a ruled stage micrometer, it will be observed that while the lines in the center of the picture are straight and distinct in outline, as they approach the periphery they become more or less curved and blurred. If we now focus downward slightly, the central lines will become indistinct and those at the edges plainer and less curved.

If our objective is well corrected for spherical aberration, the marginal lines only will be affected, and with

proper focusing will be shown with almost the same degree of clearness as the central lines originally were. If we are unable by adjusting the focus to obtain a sharp picture of the outer portion of the image, the objective may be considered to be poorly corrected for spherical aberration, and this is a grave defect.

It is not possible to entirely correct spherical aberration, and therefore the field of no objective is entirely flat. Spherical aberration is in inverse ratio to the angular aperture, and is increased with the power of the objective, and we should take these points into consideration before condemning an objective.

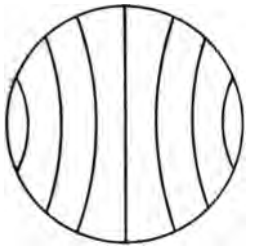

FIG. 15.

To obtain the best image possible, the object must be brought to the center of the field.

Working Distance.

By this is meant the distance between the external surface of the front lens of an objective and the top of the object viewed. As in all objectives there is a slight projection of a metal mounting beyond the lens, and as generally a thin piece of glass is used to cover the object, the *practical* working distance is thus reduced. Broadly speaking, the working distance decreases with the increase in power and aperture, but the assertion does not hold entirely good, as the optician may, within limits, overcome the difficulties set by these conditions.

In low powers the working distance is always quite sufficient for practical purposes, but in selecting the higher powers one should always pay close attention to securing a lens that will permit of the use of a cover-glass of medium thickness. The working distance of an objective is always

considerably less than the focal distance of a simple lens of the same power.

Eye-piece or Ocular.

The eye-piece occupies the upper end of the tube and receives the rays which come from the objective and conducts them to the eye. The eye-piece serves to increase the magnification of the image which we see by so refracting the rays which it receives from the objective that they form an enlarged image *within* the tube at the virtual focus of the eye-piece.

Eye-pieces are of two forms—positive and negative.

A **negative** eye-piece is one in which the focus is within the eye-piece at the diaphragm.

A **positive** eye-piece is one in which the focus lies just below the lower or field lens.

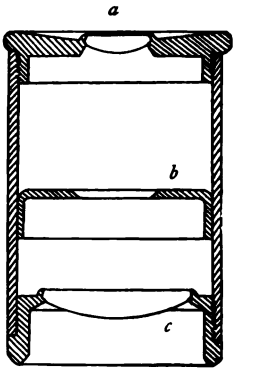

FIG. 16.

Negative eye-pieces are most commonly used. Fig. 16 shows the construction of a negative eye-piece of the Continental type. The upper *a* is called the *eye lens* and the lower *e* is called the *field lens*. At *b* is the perforated disc or diaphragm, and here the real image is formed.

The image is magnified again by the eye lens, so that we see a virtual image. This is best seen by reference to Fig. 17, in which *o g* equals the objective and *f l* the field lens of the eye-piece. Rays passing from the object *a b* through *o g* and *f l* form an image at the diaphragm *c d*. The rays from this image pass on through the eye lens of the ocular and meet the eye, which perceives at *e f* a virtual image much larger than that at *c d*.

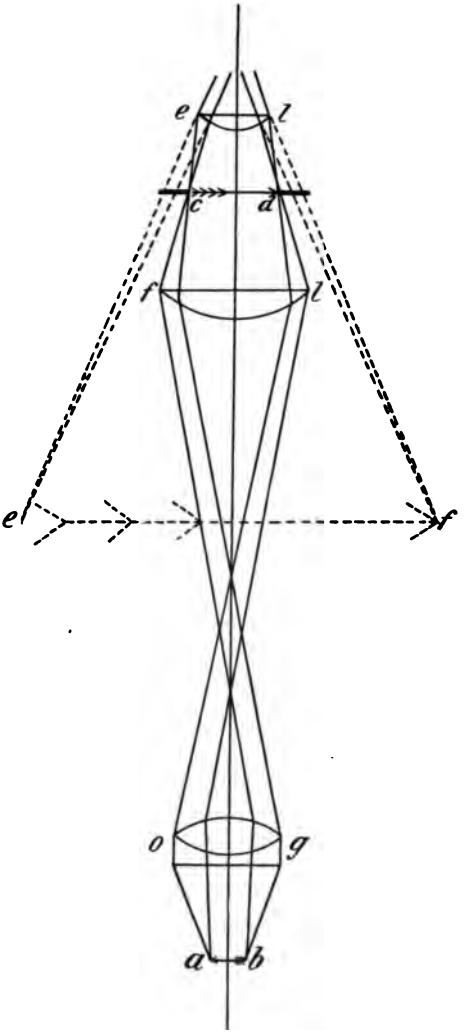

FIG. 17.

By unscrewing the eye lens and placing on top of the diaphragm a small piece of oiled paper the image here formed may be seen.

Compensating eye-pieces have already been spoken of in connection with apochromatic objectives.

Mirror.

The microscope is provided with a double mirror swinging beneath the stage. One side of the mirror is flat or plane, the other side is concave. The mirror is for the purpose of reflecting light through the object into the objective. In this case the object must be thin and transparent. If the object is opaque, it may be viewed by reflected light thrown upon it from above by a mirror or other device.

Substage Condenser.

For the high powers it is not possible to obtain sufficient light by the use of the mirror alone, and the form of substage condenser devised by Professor Abbe of the firm of Zeiss is in general use. This consists of a series of lenses held in a mounting which fits into the substage ring.

The lenses collect the light from the mirror and focus it so that the cone of light falls upon the objective, this cone having approximately the same N. A. as the $\frac{1}{12}$ objective in general use. By the use of the Abbe condenser we are able to better control the light, and while with low powers the condenser is not necessary, it is quite essential when we come to the use of objectives above the $\frac{1}{8}$ inch, and is indispensable with a $\frac{1}{12}$ inch objective.

Two forms of Abbe condenser are made: one (Fig. 18) of two lenses, which has an angular aperture of 1.20; the other (Fig. 19) of three lenses, having an angular aperture of 1.42. The one of 1.20 aperture is generally used, it being

cheaper and answering the purpose for ordinary work. An achromatic Abbe condenser of an aperture of 1.0 is also made, and may be used with advantage in photomicrography. To obtain the full angular aperture of the Abbe condenser it is necessary to use it as we would an immersion objective. A drop of cedar oil is placed upon the top of the condenser and the slide upon which the object is to be viewed is laid upon the stage and the con-

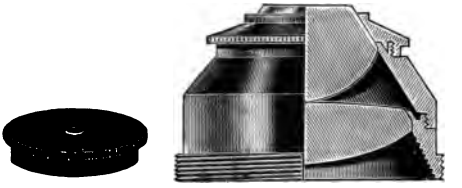

FIG. 18.
Optical part of condenser 1.20 Aperture.

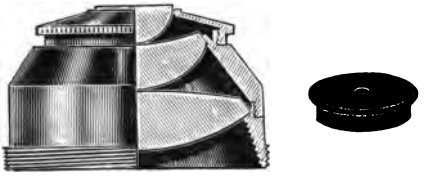

FIG. 19.
Optical part of condenser 1.42 Aperture

denser moved toward it by means of the focusing device of the substage until the drop of oil comes in contact with the bottom of the glass slide.

While this is necessary theoretically, the results obtained when using the condenser dry are so good as to make the use of the immersion fluid optional in ordinary work. It is troublesome to use it, and for this reason most workers prefer to use the condenser dry.

THE COMPOUND MICROSCOPE.

In the accompanying cut (Fig. 20) is shown the form of microscope known as the Continental, on account of this model having originated on the European continent.

This differs from the American model in three important particulars:

CONTINENTAL MODEL.	AMERICAN MODEL.
1. Horseshoe-shaped base.	1. Tripod-shaped base.
2. Tube length 160.0 mm.(6.4 in.).	2. Tube length 216.0 mm. (8.5 in.).
3. Mirror bar fixed in optical axis. (This is modified upon some stands, but there is no provision for swinging the mirror above the stage.)	3. Mirror bar may swing so as to bring mirror above stage for illumination of opaque objects

Each model has its stanch advocates. For medical and biologic use the Continental form is the one generally used on account of its compactness and general adaptability to such work.

PARTS OF THE MICROSCOPE.

The parts of the microscope as shown in the figure are:

(A) **Base.**—In the Continental form, horseshoe-shaped, lead-loaded, and resting on three points.

(B) **Pillar.**—An upright column firmly fastened to the base and supporting the remainder of the microscope.

(C) **Arm.**—This includes the parts for adjustment by micrometer screw, rack, and pinion, and supports to the body.

(D) **Body.**—The tube which carries the optical parts and the movements of which are actuated by the adjustments.

(E) **Nose-piece.**—An attachable part which carries the

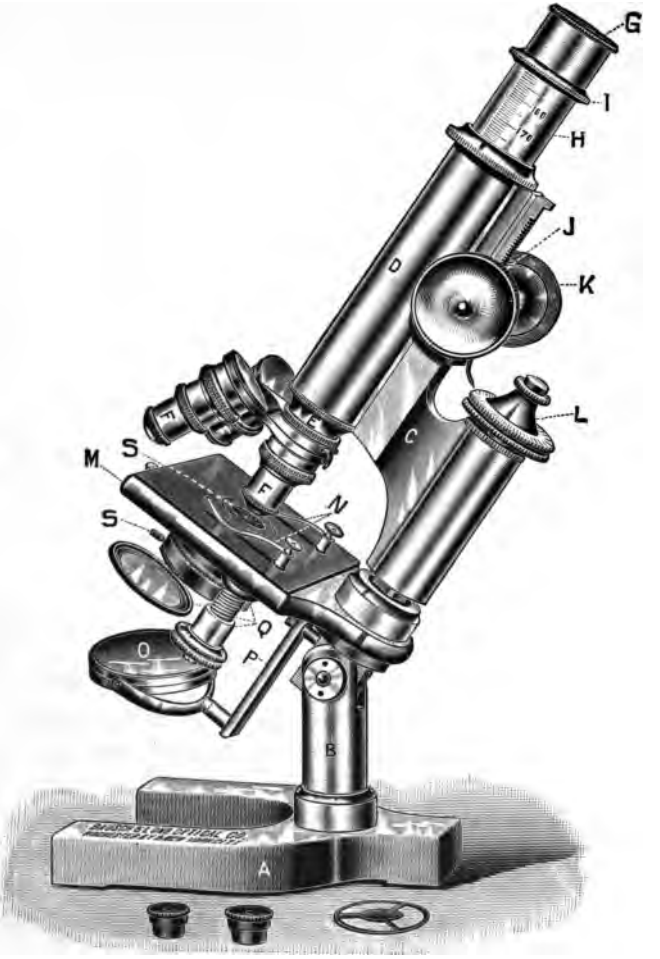

FIG. 20 —THE COMPOUND MICROSCOPE.

objectives; it may be double, triple, or quadruple. The triple is generally used. The nose-piece revolves, and one is thus enabled to turn into place either objective at will.

The Society screw or universal screw for objectives and nose-pieces is now used by all microscope makers. This is a thread of standard pitch and caliber established by the Royal Microscopical Society of London.

The objective should be so fitted to the nose-piece that if an object be focused with the low power and the higher powers be then turned into place, they will be approximately in focus, a turn of the fine adjustment being all that is necessary to give a clear image. The objectives are then said to be *par-focal.*

(F) **Objective.**—This is screwed into the nose-piece and contains the optical parts which make the first image. It is of the utmost importance that the objective be of good quality, as upon it depends in large part the perfection of the final image.

(G) **Eye-piece or Ocular.**—The optical part nearest the eye which receives the image from the objective and again magnifies it.

Oculars should be *par-focal;* that is, when one is removed and another of different magnifying power is inserted in the tube, there should be no necessity for refocusing if the object was in focus with the first eye-piece.

(H) **Draw-tube.**—An inner tube which slides up and down within the body and carries the eye-piece at its upper end. The lower end is generally fitted with a Society screw into which an objective may be screwed if one of very low power is desirable, or which may be used to hold an amplifier or other apparatus.

(I) **Collar.**—A ring united with the draw-tube.

(J) **Coarse Adjustment.**—For moving the body quickly up and down in order to bring the objective into such rela-

tion with the object that an image of the object will be formed. For this purpose most instruments are provided with a rack attached to the body and a corresponding pinion fixed upon the arm.

The coarse adjustment in the cheaper stands is obtained by means of a tube sliding in a collar attached to the arm.

(K) **Milled Heads.**—Attached to the shank of the pinion these serve to regulate the coarse adjustment of the instrument.

(L) **Fine Adjustment.**—The fine adjustment is the vital point of the microscope, and should be perfect. By turning the milled screw-head a slow and accurate movement of the arm and parts thereto attached is obtained and the relation of the objective to the object so regulated that the sharpest possible image is secured.

(M) **Stage.**—The flat projection from the arm upon which the object is placed. It may be square or round and revolving, and may also have mechanical parts for moving the object by rack and pinion.

(N) **Clips.**—Devices for holding the object in place upon the stage.

(P) **Mirror Bar.**—This holds the mirror below the stage and may be pivoted so as to permit lateral motion of the mirror.

(O) **Mirror.**—Plane upon one side, concave upon the other. The plane side should *always* be used in connection with the Abbe condenser, as by using the plane mirror more light and more perfect illumination are obtained.

(Q) **Substage.**—That part below the stage carrying the substage ring and other devices. The form of the substage may vary greatly. On the better microscopes it is provided with a rack and pinion adjustment for focusing the Abbe condenser. In many stands this movement is

obtained by means of a rapid screw placed at the left of the substage.

(S) **Diaphragm.**—The iris diaphragm has superseded all other forms. It consists of a number of metal leaves actuated by a lever, so that any desired diameter of opening may be obtained.

(T) **Joint for Inclination.**—This is now provided in all of the higher class microscopes. It is not only a convenience, but in certain work a necessity, and one should never purchase a microscope that can only be used in the upright position.

CHOICE OF MICROSCOPE.

There are many manufacturers now making good microscopes. Among the best of these are the instruments turned out by Carl Zeiss, of Jena, E. Leitz, of Wetzler, Reichert, of Vienna, Germany; Powell & Leland and Ross in England; and in this country the Bausch & Lomb Optical Company, of Rochester. This latter firm now makes a microscope that leaves little to be desired. After an experience that the writer believes should entitle him to an opinion, he does not hesitate to say that the brasswork of the microscopes made by this firm are superior to that of stands of foreign manufacture, while the optical parts are certainly as good.

For general medical work one should have a stand equipped with a substage, an Abbe condenser, a triple nose-piece, $\frac{2}{3}$ and $\frac{1}{6}$ dry and $\frac{1}{12}$ oil-immersion objectives, two inch and one inch eye-pieces. The objectives should be of medium angle. While a higher angular aperture will give the better definition, it will not have the penetration, and will probably have a shorter working distance than an objective of the same power but of lower angle.

On this account the objectives of medium angle are more satisfactory for general work. If one intends doing blood work or studying bacteria under the microscope, the $\frac{1}{12}$ inch oil-immersion lens is essential.

If unfamiliar with the microscope, the intending purchaser should always follow competent advice before selecting his instrument, as otherwise he may find too late that he has an instrument either of faulty construction or of such a model as to be unsuited to the work for which he desires it.

MANIPULATION OF THE MICROSCOPE.

One has said "Experience is the best teacher," and this is doubly true of learning the skilful manipulation of the microscope. There are a few cardinal points that the beginner will do well to bear in mind, and which may be laid down for him, but it is only by that knowledge born of labor and time that he will become expert.

Sources of Light.—Daylight is by far the preferable light for general work. One should, if possible, select north light, as this is the purest. The table should be near the window and should be of such a height as will allow one to use the microscope in the vertical position without undue effort.

Method of Illumination.

The beginner will do well first to take out the eye-piece, turn on the low power ($\frac{2}{3}$ obj.), open to the full extent the iris diaphragm, and then with the mirror bar in line with the body of the instrument tilt the mirror so that it will cast a bright beam of light into the objective, watching meanwhile through the open end of the draw-tube for this effect.

When a good beam of light is obtained, the eye-piece should be replaced and the object placed upon the stage in the optical axis.

After a little practice it will be found unnecessary to remove the eye-piece as above.

If the reflection from a white cloud can be secured, it is the ideal light. *Sunlight should be avoided, as except in expert hands it is entirely unsatisfactory.*

Focusing.—There are two methods of focusing. Until one becomes familiar with the microscope the following one should be used. Selecting the low power (⅔ obj.),—for the object should always be first examined with the low power,—the coarse adjustment is used to lower the body gently until the point of the objective just misses touching the preparation. One must use care in order to avoid forcing the objective upon and perhaps through the preparation, thus spoiling the specimen and perhaps damaging the objective. Then while looking through the eye-piece the tube should be slowly racked *up* by the use of the *coarse adjustment* until an image of the object is seen. The moment the magnified image becomes clear and distinct, movement by the coarse adjustment should be discontinued. The fine adjustment is now used until the clearest possible image of the object is obtained.

Never use the *fine* adjustment until an approximate focus shall have first been obtained by use of the *coarse adjustment.*

Modifying the Light.—It will now be seen that the object is still not distinct and has a milky look. This is because it is flooded with light and its finer details thus rendered imperceptible. This effect is easily overcome by closing the iris diaphragm until so much light is cut off as may be deemed desirable.

There can be no rule laid down for this. Each object

is a law unto itself, and one must use his judgment as to its proper illumination.

With the low power ($\frac{2}{3}$ obj.) one will need to close the diaphragm almost entirely, with the medium power ($\frac{1}{6}$ obj.) more light will be needed, while with the high power ($\frac{1}{12}$ obj.) one will often find it advantageous to use the full aperture of the diaphragm.

Always use the diaphragm wide open when viewing stained bacteria with the $\frac{1}{12}$ objective, as it renders them more distinct and the flood of light drowns out other objects that may be present.

While the above directions apply to stained objects such as are used in the examinations of tissues and bacteria, if one wishes to examine any clear fluid, such as urine for deposits, or desires to observe unstained bacteria, as in the hanging-drop, of course the light must be modified considerably, only enough being used to make objects clearly visible.

If a flood of light be turned on such objects, they being almost transparent will be quite invisible.

Care of the Microscope.

The microscope should as far as possible be protected from dust, nor should it be allowed to stand in the hot sun, as this may affect the cement of the lenses.

Before using the instrument it is well to wipe off the eye lens of the eye-piece and to see that the front of each objective is clean. "Lens paper" for the purpose of cleaning these parts may be bought from dealers in microscopic supplies, and is best adapted for this work, as a fresh piece may be used each time, obviating the possible danger of scratching the lenses with cloths or chamois, which are sometimes recommended and are apt to be dusty and unsuitable.

Never use alcohol in cleaning any part of the microscope. If balsam or cedar oil has gotten either on the lenses or lacquer of the stand, it may be quickly removed by the aid of a clean, soft cloth moistened with xylol, benzine, or benzole.

After using the oil-immersion lens it should always be cleaned by the use of lens paper before the instrument is put away. If oil becomes dried upon the end of the lens, one of the above agents may be used to soften it.

A glass bell-jar of sufficient size to cover a microscope may be procured and the instrument kept under this when not in use. One may also use a cardboard cone of home manufacture for the same purpose. It is wonderful how much hard usage a microscope may stand and still be in good working condition, but, as with any instrument of precision, it is better to take as good care of it as circumstances will permit.

If we keep our lenses free from dirt and scratches, we may expect better work from them.

The sliding parts of the body and the bearings of the pinion may every few months be lubricated with a minute quantity of clock oil. The working parts should always be kept free from dust. When it is necessary to oil the instrument, first thoroughly clean the working parts with a cloth dampened with benzine or xylol. *Never use alcohol for this purpose.*

HOW TO LOOK THROUGH A MICROSCOPE.

There is a way *not* to look through a microscope, and unfortunately this method is usually adopted by beginners.

The proper way is as follows, and it is very important that one should from the beginning accustom himself to

the rules laid down, for the sake of his comfort, for the increased facility with which he will work, and for the protection of his eyes.

First: The light should be good and not too brilliant. This can be controlled by the use of the diaphragm and mirror.

Second: The height of the table and chair should be such as will admit of looking into the microscope when it is vertical without undue bodily effort. The eye should come over the tube by simply inclining the head. There should be nothing about the neck that will when the head is bent act as a constricting band, as a high collar might do, for this will affect the circulation of the head and produce congestion of the eye, and, if long continued, the evils that may arise from this cause.

Third: Use both eyes open. This habit is easily cultivated. One at first experiences some difficulty in seeing the image in the microscope because of the confusion arising from the image seen with the other eye. A little practice will soon overcome this. Tilting the head to one side so that the nose cuts off the external image we wish to *forget* is one means of attaining the desired end. After a time the brain will take no account of the image cast upon the retina of the eye not applied to the microscope, and one may even train himself to the point where he can look in the microscope with one eye and draw the image there seen, watching the pencil point with the other eye.

Fourth: Accommodation should be thoroughly relaxed, as if one were looking at a distant object, and the focusing be done *not with the eye*, but with the fine adjustment of the microscope. The eye should not grow more tired from microscopic work than from other use.

One must remember that the ciliary muscle *is* a muscle, and is subject to the same laws as other muscles, and if it

is contracted to see that which is really out of focus, it must invariably grow tired in a short time and relax. The image will then become blurred and the observer will be obliged to rest his eye before he can proceed.

Fifth: When at work with the microscope *always keep one hand on the fine adjustment,* and while moving the object about keep up a slight to and fro movement of the fine adjustment. In this way one brings into focus objects lying in different planes which otherwise would not be seen and relieves the eye from an effort to focus upon the preparation which must necessarily be constantly occupying a different relation to the true focus of the objective.

PREPARATION OF TISSUE.

It is obvious that tissues must be prepared in some way before they can be studied under the microscope.

It is important that we see them in as nearly a normal condition as possible. We must, therefore, obtain them as soon after death as possible, whether it be death of the whole or only part of the body, as in an operation for tumor.

We must then use some chemical agent which will prevent the tissues undergoing minute changes due to decomposition or other causes.

This step is known as **fixation,** and upon the successful **fixing** of a tissue depends its value for study.

If for any reason fixation of the cells of a tissue has been poor, we cannot see it in the state in which it was during life, and may be misled in our deductions.

FIXATION.

As chemical agents will not in a short time penetrate deeply into the tissues, they must be cut in thin pieces so that the reagent may reach and kill the cells and coagulate their proteids as soon as possible.

The size of the piece of tissue must depend upon the fixing agent used and the future method to be employed. It is better to err on the safe side and not cut the blocks of tissue larger than half an inch in one diameter and a quarter of an inch thick. The selection of the fixing agent depends also on the object in view and the method of staining to be used. For routine work one may use either of the following.

Absolute Alcohol.—This is one of the best fixing agents. The tissues should be placed in it without having been previously washed in water. For a piece of tissue the above size, an ounce of the alcohol should be used. It is well to place in the vessel a quantity of cotton upon which the specimen may be laid, or in some other way to keep it in relation with the upper part of the fluid, for the reason that as the alcohol abstracts water from the tissues the lower portion of the alcohol becomes diluted, and we wish to keep the specimen in the stronger portion.

After a few hours (six or eight) the alcohol should be changed.

The tissues may remain in the second alcohol for a few days without damage.

Synthetic Alcohol.—Recently there has been put on the market an alcohol made synthetically that is claimed by the manufacturers to be equal in every respect to the distilled absolute alcohol, and which in my hands has given very satisfactory results as a fixing agent, so that for routine work, I now use no other.

Commercial Alcohol.—This is supposed to be 95 per cent. strength, generally it is about 88 per cent. It is largely used as a fixing agent, and is a fairly good one. It should be used in a little larger quantity than the absolute alcohol, but in the same way. The strength of alcohol may be preserved or increased by the use of some agent having a strong affinity for water. If several lumps, as large as a walnut, of fresh, unslaked lime be dropped into a bottle of alcohol, the water present in the alcohol and that taken from the tissues in the process of fixation will be appropriated by the lime, which will gradually slake and crumble. Sulphate of copper which has been heated till the water in it has been driven off, when it will turn *white*, may be used instead of lime. As the copper salt takes up water, it will regain its blue color, when it may again be heated and used the second time.

Formalin.—By formalin is meant a 40 per cent. aqueous solution of formaldehyde gas. It is sold under a variety of trade names. Formalin may be used diluted with water in from 2 to 10 per cent. strengths of the original solution. It is the best fixing fluid for the eye. For general tissues it is not so good as some other fluids. It has the property of keeping tissues well indefinitely, whether the fluid be frequently changed or not.

It does not bleach tissues to the extent that alcohol does, and is probably the best fluid at our disposal for the preservation of museum specimens.

Müller's Fluid.—This fluid has both advantages and disadvantages, but may be reckoned among the best fixing agents. It is composed of the following ingredients:

Potassium bichromate,	2.5
Sodium sulphate,	1.0
Water,	100.0

It may be made in quantity, as it will keep indefinitely.

Pieces of tissue fixed in Müller's fluid may be somewhat larger than those fixed in alcohol. Proportionately larger quantities of Müller's fluid must be used, and the fluid must be changed after the first few hours and upon each succeeding day during the first week, and after this as often as the fluid becomes cloudy.

The tissues must remain in the fluid until they become quite firm. If they are not too large, this will be in from two to three weeks. They must then be washed in running water for ten or twelve hours and preserved in 80 per cent. alcohol.

The great disadvantage of Müller's fluid is the time occupied in fixing tissues by it. The results obtained are very good, and for some staining methods it is necessary to use it.

DEHYDRATION.

The water normally contained in tissues must, after fixation, be abstracted before the usual infiltration processes can be used.

If absolute alcohol or synthetic alcohol has been employed as a fixative and the tissue has been put from the first solution into a fresh solution of the alcohol, it should remain here for twelve hours, and then is ready for the next step. If 95 per cent. alcohol is used as a fixing fluid, for subsequent dehydration the tissue should either be finally transferred to absolute alcohol, or, if the celloidin method is to be used, put into the third fresh solution of 95 per cent. alcohol.

If the fixing fluid has been of a watery nature, then the tissue must be run through at least three changes of 95 per cent. alcohol, remaining in each twelve hours or more, and if the paraffin method of embedding is to be used, finally put into absolute alcohol for twelve hours.

INFILTRATION.

We must in some way cut thin sections of tissue and prepare them by staining before they are fit for examination.

Tissues are normally springy, and even when well hardened cannot be cut thin enough unless they have some sort of support.

The first to be described is the celloidin method.

Celloidin is refined collodion, and comes in granular form and also in shreds. Two solutions of celloidin are made and kept in stock in tightly stoppered bottles. The celloidin is dissolved in equal parts of sulphuric ether and absolute alcohol (95 per cent. will answer, but is not as good). Solution No. 1 should be of the consistency of *thin* syrup. Solution No. 2 should be of the consistency of *thick* syrup.

The tissue should be put directly from the last alcohol into a solution of equal parts of alcohol and ether (95 per cent. alcohol may be used). Here it should remain for some time, preferably for twenty-four hours. It should then be transferred to celloidin solution No. 1, enough being used to cover the tissue well. A small wide-mouthed bottle is the most convenient receptacle, and of these, vaselin bottles of various sizes are perhaps the cheapest and easiest obtained. In solution No. 1 the tissue should remain at least twenty-four hours, unless the pieces be very small. It may remain in this solution for several days with advantage. It should then be transferred to celloidin solution No. 2, where it should remain twenty-four hours or longer.

MOUNTING.

The tissue must now be mounted upon a stable support capable of being held firmly in the jaws of the microtome. Cork is largely used and is recommended in some text-books. On account of its springy nature cork is entirely unsuitable, and should never be used for this purpose.

The author uses pieces of hard wood,—maple or black gum,—having a flat circular head 1¾ inches in diameter, ¼ inch thick, and with a central stem 1¼ inches in length and ⅜ inches in diameter (Fig. 21). These mushroom-shaped pieces of wood have many advantages, and any carpenter will turn them out on his lathe at a nominal cost.

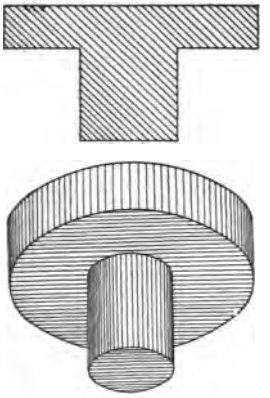

FIG. 21.

The tissue, now thoroughly infiltrated with celloidin, is removed from the celloidin solution No. 2 with a pair of forceps and placed upon the flat surface of the wooden support. Enough celloidin is poured around it to form a ring of this substance, which when hardened will serve to hold the tissue firmly in place upon the support. Within a few moments it will be found that the evaporation of alcohol and ether from the celloidin has caused the part exposed to the air to become firm, so that there is a thick skin formed, sufficient to keep the still fluid portion below in place. The block of wood with its tissue should now be transferred to a jar of 80 per cent. alcohol.

The block should be placed in the alcohol tissue-side down and allowed to remain twelve hours. The alcohol will harden the celloidin both around and in the tissue,

giving the whole mass quite a firm consistency, which will readily admit of thin sections being cut from it. The specimen may be left in the 80 per cent. alcohol indefinitely.

If one is in a hurry to secure a section, chloroform may be used as a hardening agent for the celloidin. Chloroform will harden celloidin in about half an hour. The specimen may then be preserved in 80 per cent. alcohol.

THE PARAFFIN METHOD.

Paraffin may be used as an embedding material, and for certain work is better than celloidin.

Paraffin is especially valuable for making serial sections, as in embryologic work. With it large and thinner sections may be cut. Paraffin embedding is generally used for cytologic work on account of the thinness and uniformity with which sections may be made. It has the additional advantage of preserving the block of tissue indefinitely. The paraffin should thoroughly infiltrate the tissue, so that it will universally support it.

The preparation of tissue for the paraffin method is the same as for the celloidin method, with one exception—*absolute alcohol* must be used in the last steps of dehydration. As absolute alcohol is expensive, it is the author's practice to use two or three changes of 95 per cent. alcohol in dehydrating tissue fixed in aqueous fluid, and to finish dehydration in one or more changes of absolute alcohol. If possible, the tissue should remain in each of the various alcohols for at least twelve hours. With pieces of tissue no larger than 1 cm. square the process may be somewhat hastened.

The tissue is now transferred to a small jar or wide-mouthed bottle containing *chloroform*. If thoroughly de-

hydrated, the tissue will float in the chloroform. If not dehydrated, the tissue will not float or will scarcely do so, and there will be given off from it a milky cloud which will be very perceptible in the clear chloroform. If this milkiness is observed, the tissue must at once be put into fresh absolute alcohol and allowed to remain there at least twelve hours. It may then be tried again in chloroform, and will probably be found to be dehydrated.

If the above directions are disregarded and tissue which is not thoroughly dehydrated is carried through the paraffin, it will be found that it is entirely unfit for sectioning and study. One should therefore "make haste slowly."

Infiltration with Paraffin.

Paraffin is solid, and it is necessary to reduce it to a liquid state before it will enter the tissue. To do this we must resort to heat. This is unfortunate, because too high a degree of heat may produce changes in the tissues, and we must have a special apparatus in which we can melt the paraffin. In the catalogues of the dealers are found descriptions of the various kinds of paraffin baths. The writer much prefers the ordinary water-bath (Fig. 22). Not only is it more cleanly, but it provides a more uniform heat and may be used for other purposes. A thermostat must be used in connection with it.

The melting-point of paraffin varies; in winter a soft paraffin may be used, while in summer it is necessary to use a hard paraffin. A paraffin having a melting-point of 54° C. will generally answer for ordinary work.

When one wishes to do the fine sectioning required in cytologic research or in cutting small embryos, the melting-point of the paraffin must be chosen with reference to the room-temperature at which the sections are to be cut.

Two paraffins should be used.

Paraffin No. 1 should have a melting-point of about 44° C. A quantity of this soft paraffin is cut into small pieces and placed in a suitable receptacle—an ordinary teacup answers for this purpose—and melted in the water-bath. The block of tissue is transferred from the chloroform to the paraffin and allowed to remain in the water-bath twelve hours. It is then transferred to the melted paraffin No. 2.

Paraffin No. 2 is pure paraffin having a melting-point of, say, 54° C. Tissue should remain in No. 2 in the water-bath twelve hours, and then is ready for embedding.

FIG. 22.

Embedding in Paraffin.—The block of tissue is now thoroughly impregnated with the harder paraffin. It must next be embedded in a mass of paraffin which will act as a support to the margins of the tissue and serve to fasten it to the wooden block.

The tissue is removed from the melted paraffin with a pair of forceps and placed upon a piece of glass, that part which it is desired to be cut *first* being placed in contact with the glass. The tissue is then surrounded by a wall of some substance that will act as a mould. A piece of paper may be bent to answer this purpose, or brass or lead piping of various diameters cut in short pieces answers very well.

Moulds made of rectangular pieces of type metal are

perhaps the best, as with these we secure a square block, and the size of the block may be varied by adjustment of the sides of the mould. Such embedding boxes may be bought in the shops (Fig. 23). The mould is now filled with melted paraffin No. 2 and the whole cooled as rapidly as possible to prevent crystallization of the paraffin. This may be accomplished by placing the glass plate in cold water, being careful not to allow the water to run over the mould until the surface of the paraffin has cooled.

When the paraffin block is thoroughly hardened, it is removed from the mould, and may be cut immediately or at a convenient season.

Mounting the Paraffin Block.

When it is desired to section the tissue, the block of paraffin is fastened to a piece of wood that may be clamped in the jaws of the microtome. This is best accomplished by heating the blade of a case-knife in the flame of a bunsen burner or spirit-lamp until it will melt the paraffin.

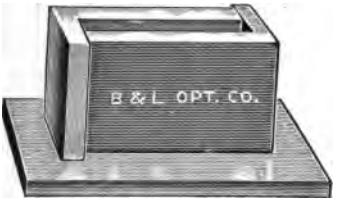

FIG. 23.—PARAFFIN EMBEDDING BOX.

The heated knife-blade is placed flat upon the wooden support and the bottom of the paraffin cast brought in contact with the knife-blade immediately over the block. When the paraffin begins to melt, the knife is slipped quickly from beneath it and the cast and support pressed firmly together. In a few minutes the paraffin will have cooled and the cast is firmly fixed to the support.

The mushroom-shaped pieces of wood spoken of in connection with the celloidin method will be found equally adapted to paraffin work. It is often found advantageous to place the tissue eccentrically upon the block, as this

facilitates the proper adjustment of the paraffin cast in its relation to the knife-edge.

TABULATION OF STEPS IN CELLOIDIN METHOD.

1. Fixation.—Absolute alcohol or synthetic alcohol or 95 per cent. alcohol, or formalin in 10 per cent. strength, or Müller's fluid.
2. Dehydration.—*If fixed in absolute alcohol,* into the fresh absolute alcohol for twelve hours.
   If fixed in 95 *per cent. alcohol, into:*
   (*a*) 95 per cent. alcohol No. 2 for twelve hours.
   (*b*) 95 per cent. alcohol No. 3 for twelve hours.
   If fixed in watery solution, into:
   (*a*) 95 per cent. alcohol No. 1 for twelve hours.
   (*b*) 95 per cent. alcohol No. 2 for twelve hours.
   (*c*) 95 per cent. alcohol No. 3 for twelve hours; or, better, in absolute alcohol for twelve hours.
3. Ether and Alcohol.—In ether and alcohol equal parts for twelve hours.
4. Thin Celloidin.—Celloidin consistency *thin* syrup twenty-four hours, or longer.
5. Thick Celloidin.—Celloidin consistency *thick* syrup twenty-four hours or longer.
6. Mount.—*On wooden support.*
7. Alcohol.—Harden celloidin and preserve in 80 per cent. alcohol. (Chloroform may be used.) May be cut after twelve hours.
8. Sectioning.

TABULATION OF STEPS IN PARAFFIN METHOD.

1. Fixation.—Absolute alcohol or synthetic alcohol or 95 per cent. alcohol or formalin 10 per cent. or Müller's fluid.
2. Dehydration.—Must be thorough.
   If fixed in absolute alcohol:
   (*a*) Absolute alcohol No. 2 twelve hours.
   (*b*) Absolute alcohol No. 3 twelve hours.
   If fixed in 95 per cent. alcohol:
   (*a*) 95 per cent. alcohol No. 2 twelve hours.
   (*b*) 95 per cent. alcohol No. 3 twelve hours.
   (*c*) Absolute alcohol twelve hours.
   If fixed in aqueous solution:
   (*a*) 95 per cent. alcohol No. 1 twelve hours.
   (*b*) 95 per cent. alcohol No. 2 twelve hours.
   (*c*) 95 per cent. alcohol No. 3 twelve hours.
   (*d*) Absolute alcohol twelve hours.

3. Chloroform.—For twelve hours. Tissues should float and not turn chloroform milky. Failure in this indicates insufficient dehydration.
4. Infiltration.—Paraffin No. 1: Soft paraffin. Melting-point of paraffin 44° C. Tissue kept in melted solution in water-bath twelve hours.
   Paraffin No. 2: Hard paraffin, melting at 54° C. in water-bath at temperature just sufficient to keep melted twelve hours.
6. Embedding.—On glass plate in mould.
7. Mounting.—On wooden support; adhesion secured by melting bottom of cast.
8. Sectioning.

SECTION CUTTING.

The microtome is an instrument designed to cut pieces of tissue thin enough for microscopic examination.

There are a variety of forms of microtomes upon the market, but two chief principles are employed: the one, in the Thomer model, in which the tissue is fed upward by being advanced on an inclined plane; and the other, in the Schanze model, in which the tissue is fed upward by a micrometer screw. In either model the knife is carried on a sliding block and propelled by the hand.

Each form of instrument has its merits, but mechanically the Schanze model is the more perfect instrument. It is also capable of much more rapid manipulation.

In this country the Bausch & Lomb Optical Company, of Rochester, makes a very good line of microtomes, and from their catalogue one may make a selection according to the work one desires to do and the money one has to expend.

The student microtome (Fig. 24) is a very serviceable instrument for individual work. It is unsatisfactory for large sections; and if much sectioning is contemplated, a larger instrument should be secured.

FIG. 24.

It is not possible to get one microtome that will meet every requirement.

Serial sectioning of small embryos of other objects can best be done upon the microtome designed especially for the work. The best microtome of the class is the Minot. It is quite expensive, however, and the Ryder microtome, manufactured by Zentmeyer, of Philadelphia (Fig. 25), will be found to be a very efficient instrument, not only for serial sectioning, but for general paraffin work. It is

FIG. 25.

small, compact, and cheap, a very serviceable model and deserving of general use. Unfortunately, it is not well made.

Care of the Microtome.—While the microtome is not a very delicate instrument, it will do better work if properly cared for. It should be kept covered when not in use, and the sliding parts should always be cleaned before using by wiping them with a cloth. They should then be oiled with a drop or two of paraffin oil, which has proved more

satisfactory in the writer's hands than any of the lubricants generally used, because it does not gum and harden. The micrometer screw should also be frequently cleaned and oiled.

The Knife.—One who possesses a good microtome knife is fortunate. It must be of just the proper temper or it is a failure, and it does not seem possible for the manufacturers to with certainty make two tempered alike. At any rate, the purchase of a microtome knife is a lottery.

The size of the knife must correspond to the size of the microtome upon which it is to be used. The larger the knife, the better the results it should give, as there will be less spring to cause deviation in the thickness of the sections.

Care of the Knife.

One cannot be too careful of his microtome knife. Its edge must be perfect or it will not cut thin sections. It must never be left upon the microtome when work is done. For the time, remove the knife, thoroughly cleanse, and put it in its case. If paraffin is caked upon it, use a solvent—xylol or chloroform—to remove it. Never touch the edge with any hard substance.

Sharpening.—One must learn the art of sharpening a microtome knife by practice. At first he will succeed but poorly. Two hones should be kept for the purpose—a yellow Belgian water-hone and a slate water-hone. The first is used only occasionally, when the edge is very dull or has become rounded or nicked. In other words, this stone has a sharp tooth and is only serviceable when we wish to cut away metal rapidly.

The blue hone cuts very slowly and leaves a polished, fine edge. The lower surface of the knife is made flat or very slightly concave, and when the knife is on the micro-

tome should lie nearly on a level with the plane of the ways upon which the carrying block slides, there being a slight upward tilt of the back of the knife so that the blade does not touch the block of tissue except on the cutting-edge.

The upper surface of the blade is slightly concave.

In sharpening the knife the bottom surface should be laid flat against the stone.

The upper surface is sharpened quite differently, being held so that the edge will be worn away at an angle of about 15 degrees.

Fig. 26 is the cross-section of a knife as it should appear when properly sharpened.

In honing, the knife should always be drawn from the

FIG. 26.

heel to the point, the sharpening being done largely from the upper, beveled surface, the lower surface needing only a few occasional strokes with the blade flat upon the stone.

Many workers use finally a strop backed by a flat piece of hard wood so that the edge of the knife will not be turned by the sagging of the leather. For some years the writer has discarded the strop. With a good knife and correct honing it is not necessary.

A microtome knife, when properly sharpened, should cut a hair held in the hand, as in testing a razor.

Sectioning Tissues Mounted in Celloidin.

In cutting celloidin sections the knife and the tissue should be kept flooded with 80 per cent. alcohol. One

method of keeping the knife wet is to mop the alcohol on it with a large camel's-hair brush between each stroke. This is clumsy and unsatisfactory. A better way is by means of an inverted flask of alcohol suspended over the knife. The mouth of the flask is closed with a perforated rubber stopper with a piece of glass tubing passing through it. Over the outer end of the glass tubing is passed a small piece of soft-rubber tubing of such length as nearly to reach the knife. A pinch-cock controlled by a screw clamping the rubber tube completes the device.

With this simple apparatus one may exactly regulate the flow of alcohol to the desired quantity. The alcohol should be allowed to drop in such a way that the surface of the specimen and of the knife are kept wet with it. A little practice will soon determine the quantity necessary to insure good sections.

In cutting celloidin sections the knife should be set so that it will have the longest possible sweep. In other words, all of the edge of the knife should be used in the stroke.

The tissue should generally be placed so that the knife will strike the tougher portion first. As an illustration, in cutting a piece of tumor having a capsule the capsule should be set toward the knife-edge.

As the sections are cut they must be transferred to 80 per cent. alcohol. After a few have collected upon the knife they may be removed by sliding them off the edge of the knife on to the finger placed beneath the blade and in contact with it. Some use a camel's-hair brush for this purpose. Trained fingers are more satisfactory in every way.

The sections may be indefinitely preserved in 75 per cent. or 80 per cent. alcohol. In stronger alcohol they are apt after a time to lose their staining qualities.

CUTTING PARAFFIN SECTIONS.

Tissue embedded in paraffin is always cut dry. After the block has been fastened to the support the redundancy of paraffin should be trimmed away from around the upper part of the tissue with a knife, leaving enough paraffin to act as a support to the edges.

It is not necessary in general work to have the knife at any fixed angle to the tissue. The slant at which it works best is the one to be secured, and this can be obtained only by trial and varies with the tissue to be cut.

If the temperature of the room is just right, the sections when made will remain comparatively flat. If the paraffin is too soft, the sections will crumple and stick together; if too hard, the sections will curl. The temperature may be regulated in some degree by opening or shutting a nearby window or by placing a lump of ice in the vicinity of the microtome. In spite of all we can do, the sections will sometimes be refractory.

The section flattener devised by the late Dr. James E. Reeves is a useful adjunct. It consists of a wire frame clamped to the knife and holding a piece of stiff paper in such a way that the sections as they are cut slide under it and are kept from curling. A piece of paper held in place by the hand answers the same purpose. The paper should lie in a plane with the surface of the knife and almost in contact with it.

As sections are cut they should be removed from the knife and laid on clean paper, or, if the temperature of the room necessitates it, in dishes of cool water. Paraffin sections cannot be handled with the fingers. A pair of fine-pointed forceps is useful for this purpose. Paraffin sections cannot be preserved unmounted, but the em-

bedded block of tissue may be kept for years and sections cut from it when desired.

Mounting Paraffin Sections.

The sections must now be attached to glass slips preparatory to being stained. It is necessary to use some cement to fasten the sections in place. A 0.5 per cent. solution of the best gelatin in a 2 per cent. watery solution of carbolic acid is quite satisfactory.

The slips should be well cleaned and free from dust. With a pipette a quantity of the "fixative" is put on a slip, a section is lifted with the forceps and laid upon the solution which must be in amount sufficient to float it, and the slip is warmed either in an oven made for this purpose or by passing it a few times through the flame of a bunsen burner.

As the liquid becomes warm the section flattens out perfectly if it has not been allowed to roll up tight when cut. The section is placed in the middle of the slip and the fixative drained off; when cool, the section is lightly blotted with bibulous paper and the slip set aside to dry overnight.

Generally the tissue is well attached to the glass by this method. In order to insure success, the writer is in the habit of submitting the specimen to the action of formalin, which renders the gelatin insoluble. The slips with the sections attached are put in an ordinary slide-box, a few drops of formalin are poured into the bottom of the box, and the cover is closed. When the gelatin water has thoroughly dried, staining may be proceeded with.

THE FREEZING MICROTOME.

Various forms of freezing microtomes may be bought, and in most of these the freezing agent is ice and salt. Carbonic acid gas is also used, and is preferable to ice because of the cleanliness, more rapid action, and from the fact that it is always ready.

Very good sections may be made by the freezing method, and when time is limited, as in the diagnosis of tumors during operations, it is valuable. It is not applicable to general work.

If tissues have been fixed in alcoholic solution, they must first be washed for several hours in order to remove the alcohol, else they cannot be readily frozen. Tissues, whether fresh or hardened, should be put on the freezing block and covered with a thick solution of mucilage. Do not freeze too hard or the knife may suffer damage. As the sections are cut they should be transferred to a dish of water, or, if the tissue is *unhardened*, to a 5 per cent. aqueous solution of formalin. After remaining in formalin for five minutes they will be hardened sufficiently to bear handling, and may be stained and mounted for examination after the manner of celloidin sections.

Thickness of Sections.—In general pathologic work the mistake of cutting sections too thin is very often made.

While we must have our sections thin enough to freely transmit light, we get a better idea of the relation that cells have to each other if they are moderately thick. In cytology, and frequently in bacteriologic research, it is necessary to have sections as thin as possible. One must use his judgment in this matter and cut his sections according to the end in view.

The beginner will do well to purchase a few preparations as a standard with which to compare his work.

STAINING CELLOIDIN SECTIONS.

Stains are used for the purpose of bringing out the structural details of tissues. Almost every known dye has been used in some staining method, and the mass of material in books on technique from which one must cull that which is serviceable is very confusing to the beginner.

For general work, the **hematoxylin** and **eosin** method is unsurpassed. It is simple, effective, and permanent.

There are numerous formulæ for hematoxylin stain, and each has its advocates, and perhaps all are good. A standard formula is the following:

BÖHMER'S HEMATOXYLIN.

Hematoxylin crystals,	1.0
Alcohol,	10.0
Potash alum,	10.0
Water,	200.0

Dissolve the hematoxylin crystals in alcohol, making solution A. Dissolve the alum in the water, making solution B. Add A to B, drop by drop. Place the mixture in an unstoppered bottle in a strong light to ripen. It will gradually turn dark and will be fit for use in about ten days. Filter and add a few small lumps of gum camphor to keep moulds from growing in the stain. Most of the camphor will remain undissolved. Keep in a well-stoppered bottle. The staining qualities increase with age. The stain works best undiluted and may be used over again any number of times.

Hematoxylin is a nuclear stain; that is, it stains the nuclei of cells and for the most part leaves the protoplasm of the cells unstained. Nuclei stained by hematoxylin should be a deep purple color. If they are red or brown,

the stain is not good or they have not been washed long enough.

1. **Time of Staining.**—One must use his judgment as to the length of time to stain a given section.

Newly made stain does not work so quickly or with such intensity as old stain, and there is much variation in the affinity of tissues for stain. With a well-ripened stain the process should take from one to three minutes.

Practice will soon make one expert in this matter.

2. **Staining.**—Hematoxylin stain must always be filtered before using. One should have at hand a small glass funnel kept for this purpose alone. The same filter-paper may be used many times. Sections in 80 per cent. alcohol should first be put in a dish of clear water.

Sufficient stain to cover the section well is filtered into a suitable dish or Syracuse watch-glass; the section is transferred from the water directly to the stain and immersed there for the space of time deemed necessary.

Sections are best handled with a blunt, curved needle, such as may be bought of the dealers. A common saucer with *straight* sides is the best dish for fluids used in the following manipulations.

3. **Washing.**—Transfer the section to a saucer of clean water and by gentle manipulation of the curved needle wash it free from stain. When it is freed from superfluous stain, transfer to a saucer of fresh water for five minutes or longer.

If one is not in a hurry, it is better to leave the section in the second water for several hours or even overnight.

This long washing serves to extract the stain from those parts of the cell which have not a special affinity for it and gives us "differentiation," so that a clear, sharp picture results.

4. **Dehydration.**—The water must now be gradually

extracted from the section in order that it may be cleared and mounted.

This is accomplished by putting the section first in an alcoholic solution which is weak (70 per cent.) for five minutes, and then into a stronger alcohol (95 per cent.) for five minutes, and finally into a second strong alcohol (95 per cent.).

5. **Counterstaining.** — Eosin stains the protoplasm of the cell, connective tissue, red blood-cells, and tissues which have undergone some of the degenerative changes.

It is a contrast stain and tissue renders the section more intelligible to the beginner. Many advise staining with aqueous eosin before dehydration, but alcohol being a good solvent of eosin much of the stain is lost in the latter process, and one is never quite certain how deep his section will be colored when mounted.

By using a ¼ of 1 per cent. solution of eosin in absolute alcohol (95 per cent. will answer) after dehydration one may exactly regulate the degree of staining. Two minutes in this solution is generally sufficient. Overstaining with eosin makes a muddy section and obscures fine detail.

6. **Clearing.**—In order that the section may readily transmit light and to extract the alcohol from it some clearing agent must be used. The essential oils—oil of cloves, origanum, turpentine, and others—are used for the purpose, and in some staining methods it is essential that a particular oil be employed.

The best clearing fluid for general use is carbol-xylol, and it is surprising that it is so little used.

It clears sections dehydrated with 95 per cent. alcohol, while with some of the oils it is necessary to use absolute alcohol, it does not dissolve the celloidin, and the penetration is much more rapid than with any other agent. It is also free from the disagreeable odor of clove oil and

creosote, and being very liquid and volatile is easily cleaned from the slide.

These qualities and the fact that it may be used with most staining methods, and clears the section quite as well as other fluids used, should make it more popular than it now is. The formula is as follows:

Carbolic acid,.....1 part.
Xylol,.....3 parts.

The xylol is very volatile, and, as the fluid is repeatedly used, this factor gradually evaporates and the percentage of carbolic acid becomes greater. When this occurs fresh xylol should be added in such quantity as is considered necessary.

The section is put from the alcoholic eosin into a saucer of carbol-xylol, where it at first *floats* on the surface but within a few seconds slowly sinks.

Two minutes is ample time to allow for clearing with this mixture; one minute being in most instances quite sufficient.

7. **Mounting.**—The section is now ready to be put upon the glass slip. Metal lifters are generally used for this purpose, but a piece of *cigarette* paper is far better. A strip of paper a little wider than the section to be handled is used. It should be laid against the straight side of the saucer with the lower end projecting into the fluid for a distance slightly exceeding the diameter of the section.

The latter is now floated on to the paper by manipulation with the curved needle. The paper with the section clinging to it is withdrawn by sliding it up the slanting side of the saucer, the surplus clearing fluid drained off, the paper inverted, and the section brought into contact with the center of a clean glass slip. While the paper is

still covering the section, blot the latter with bibulous paper.

The paper is now gently lifted, the section remaining on the slip. A drop of Canada balsam dissolved in xylol is put upon the section and the whole covered with a clean cover-glass of proper size. The cover-glass should be tightly pressed down upon the section with the butt of the forceps. The **slide,** as the finished specimen is called, is now ready for inspection with the microscope.

STAINING PARAFFIN SECTIONS ON THE SLIP.

The sections being mounted on pieces of glass 1 × 3 inches, it is necessary in order to bring them in contact

FIG. 27.

FIG. 28.

with the various reagents to have some form of dish that will admit of the slips being stood on end in the fluid. The **Stender dishes** (Fig. 27) or the **Naples staining jar** (Fig. 28) are adapted to this purpose.

The paraffin must first be gotten rid of. This is accomplished by immersing the slip in a dish of xylol for ten minutes or more. When it is removed, the surplus reagent should with a towel be wiped from the under surface of the slip and from around the section, care being taken

not to touch the latter. The slip is then placed in 95 per cent. alcohol for five minutes to take out the xylol, then in 75 per cent. alcohol for one minute, and then in water for one minute.

The remainder of the staining process is identical with that described for staining the celloidin sections except that Stender dishes must be substituted for saucers.

The slips should be wiped free from each reagent before being put into the next in order to avoid contamination of solutions.

The finished *slides* may be placed in the drying oven for several days for the purpose of hardening the balsam.

STAINING CELLOIDIN SECTIONS.

The sections are presumed to be taken from 80 per cent. alcohol.

1. Water—two minutes.
2. Hematoxylin—one to three minutes.
3. Water to wash off stain.
4. Water, clean, to differentiate, fifteen minutes or longer.
5. Alcohol (70 per cent.) five minutes.
6. Alcohol (95 per cent.) five minutes.
7. Alcohol (95 per cent.) five minutes.
8. Alcoholic eosin ($\frac{1}{4}$ of 1 per cent.) two minutes.
9. Carbol-xylol, two minutes.
10. Balsam.
11. Cover-glass.

STAINING PARAFFIN SECTIONS.

Staining on the slide.

1. Xylol ten minutes.
2. Alcohol (95 per cent.) five minutes.

3. Alcohol (75 per cent.) one minute.
4. Water one minute.
5. Hematoxylin one to three minutes.
6. Water to wash off stain.
7. Water, clean, to differentiate, fifteen minutes or longer.
8. Alcohol (70 per cent.) five minutes.
9. Alcohol (95 per cent.) five minutes.
10. Alcohol (95 per cent.) five minutes.
11. Alcoholic eosin (¼ of 1 per cent.) two minutes.
12. Carbol-xylol, two minutes.
13. Balsam.
14. Cover-glass.

Deep dishes in which the slides may be immersed in the fluid must be used.

BACTERIA.

For a long time it was thought that bacteria belonged to the animal kingdom. They are now known to be vegetable.

The term bacteria is applied to those minute and simple forms that occupy the lowest place in the vegetable kingdom.

Bacteria are classified according to their form into **round bacteria** or **cocci**,* **rod-shaped bacteria** or **bacilli**, and **spiral bacteria** or **spirilla.**

The first group is further divided into single forms, **monococci**; double forms, **diplococci**; those in which four organisms are grouped, forming a square, **tetracocci**; and **sarcinæ**, the latter occurring in bale-like masses.

* Pronounced *kok'-si.*

Single cocci occurring in bunches are called **staphylococci** (from σταφυλη, "a grape"), because in the tissues they grow in clusters or masses, like a bunch of grapes.

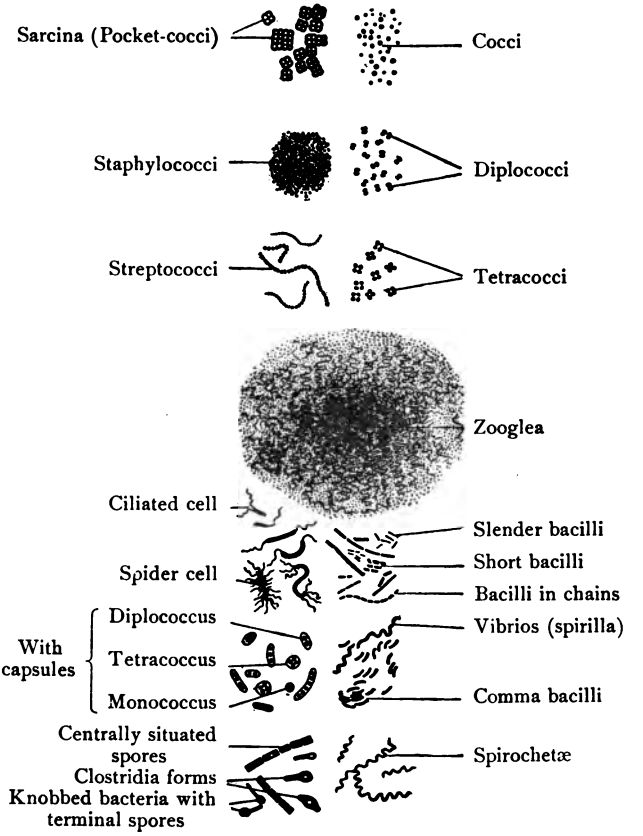

FIG. 29.—FORMS OF BACTERIA.—(*From Schenk.*)

Other cocci occur frequently in chains, and they are caled **streptococci.**

The variation in the arrangement of cocci is due to their

method of development. All cocci multiply by fission or division.

The diplococci after the division of the single cell remain for a time in apposition. Tetracocci are formed by the splitting up into four parts of a single coccus, and the sarcinæ by the division of a single coccus in a number of directions.

BACILLI.

Any micro-organism having one diameter exceeding another is called a bacillus. Some of the bacilli are so nearly round as almost to deserve classification among the cocci.

Bacilli multiply by division into pairs, this process being called **fission.** If bacilli exist in an end-to-end formation or chain, they are called **strepto-bacilli.**

Many bacilli form spores. **Spores** are minute globular bodies formed within the bacilli by a condensation of their protoplasm. The spores may be situated in the middle or end of the rod. Some bacteria may contain several spores. Spores may be likened to the seeds of the higher plants, and under favorable conditions each spore produces one bacterium.

Spirilla are rod forms that have a spiral shape. Some spirilla are long, narrow, and wavy, while others are short and thick. The spirillum of cholera is of the latter type. Multiplication of spirilla usually takes place by fission, but spores have been observed in a few species.

Bacteria are divided into two great groups, those that live normally in the living body of animals or plants being called **parasites,** while those that live in dead matter are known as **saprophytes.**

Some parasites can grow only in living matter; these are called **obligate** parasites, while those that may grow

either upon the living body or upon inert material are called **facultative** parasites. In other words, these parasites may become saprophytes.

Motility.—Some bacteria have the power of motion, and can be seen, when studied under the microscope in the hanging drop, moving across the field, often with considerable rapidity. The movements are sometimes erratic or they may be quite direct or they may be dancing.

The activity of motile bacteria must not be confounded with the motion that all small particles of matter in suspension may assume, known as the Brownian movement. In the Brownian movement the particles dance to and fro

FIG. 30.—HANGING-DROP PREPARATION.

but do not change their relations to each other nor move from the field of vision.

Bacteria move through the agency of small thread-like prolongations of their protoplasm. The tail-like appendages are called flagella, their number differing in varieties of motile organisms. A bacterium may have a single flagellum at one end or there may be several at one end, or each end may contain tufts of flagella, or the organism may have them projecting from it in every direction, in which case it presents a mossy appearance. Flagella are found on many species of bacilli and spirilla or vibrios, but only a few varieties of cocci possess them.

It is impossible to see flagella in unstained bacteria, neither do they stain by the ordinary methods. In order to see them we must use special technique, which will be given in detail later (see p. 114).

GROWTH OF BACTERIA.

There are five varying conditions necessary to the growth of bacteria: food, moisture, temperature, light, and atmosphere.

Food.—Bacteria perform the great function of breaking up organic compounds into simpler elements. We find in nature that bacteria are responsible for all decay, whether of animal or vegetable bodies. We know also that the growth of one variety may be retarded or accelerated and its characteristics changed by the presence of other varieties in the same soil.

In the laboratory it is necessary to have some food of definite composition in which to grow bacteria for experimental purposes. A number of such foods have been devised and are in common use, and these are known as **culture-media.** These culture-media are not ideal foods, but most bacteria grow fairly well upon at least some one of them.

When planted on culture-media, we find that bacteria produce a chemical substance that retards and finally stops their growth. The nutrient portion of the medium is not all used up, and yet the growth of the organisms may be limited to a small area. Generally the limit of growth is reached in forty-eight hours.

The retarding chemical substance or **enzymes** produced by one variety of bacteria are generally unfavorable to the growth of another variety.

Moisture.—Bacteria may exist for a time in the dried state, but they cannot grow without a proper amount of moisture.

Spores will live much longer in the dried state than will the bacteria themselves, but the amount of resistance to drying depends largely on the variety of the organism.

Anthrax spores in the dried state retain their vitality for several years.

Temperature is an important factor in bacteriologic growth. The temperature at which a bacterium will grow best is called the *optimum temperature*. The temperature above which an organism will not grow is called the *maximum temperature*, and the temperature below which it will not grow is called the *minimum temperature*.

Naturally, the optimum temperature for parasites is the temperature of the body they are parasitic to. In the case of bacteria inhabiting animals, the body-temperature of 37° C. is the optimum temperature. A few organisms grow best at a very high temperature, 60° or 70° C., and these are called **thermophilic** bacteria.

An organism may not be killed by being subjected to a temperature below its minimum or above its maximum temperature. Many organisms will resist freezing temperature for a long time, and will grow as soon as the proper conditions are again established. Other bacteria will resist high degrees of heat, and this is partially true of the spore-bearing organisms. A temperature above the maximum temperature of an organism does not necessarily kill it, but it stops its growth.

Pathogenic bacteria grow at a degree of heat just below their maximum temperature, but lose their virulence; and those bacteria which produce color under normal conditions fail to do so if grown at a temperature near their maximum temperature.

Light is nature's greatest germicide. Sunlight kills bacteria in even a few hours' time, and strong electric light has a similar effect. Even diffuse daylight kills bacteria if they are subjected to its influence for a considerable time. The less bacteria are protected by surrounding influences, the quicker will light affect them. Dried

anthrax spores are killed in an hour and a half by direct sunlight; but if the spores are moist, they are not killed so quickly.

Atmosphere.—Most bacteria grow only in an atmosphere of oxygen, such are called **obligate aerobes;** while others will not grow in the presence of oxygen, and these are called **obligate anaerobes.** A third class may grow either in the presence of oxygen or without it, and these are called **facultative anaerobes.**

For cultivating anaerobes special methods are resorted to, they being generally grown in an atmosphere of hydrogen.

Chromogenic Bacteria.—Many bacteria have the property, under favorable conditions, of producing color in the course of their growth. The pigment in some varieties is within the bacterium, and in other varieties is produced outside of the organism and may even vividly stain the culture-medium at a considerable distance from the area of bacteriologic growth.

The pigments of the chromogenic bacteria are generally soluble in alcohol, chloroform, or ether, and insoluble in water. The more common colors thus produced are reds, yellows, and violets. The common pus organism Bacillus pyocyaneus imparts a vivid blue color to the medium in which it grows. This may be abstracted by chloroform in the form of *pyocyanin*, which is crystallizable.

Pathogenic Bacteria.—Certain forms of parasitic bacteria are capable of producing disease or pathologic conditions of the body, and to these the term **pathogenic** has been applied.

The pathogenic organisms are the ones of most interest to the physician, and the scope of this book does not admit of other than these being considered.

BACTERIOLOGIC METHODS.

For the study of bacteria one must have certain facilities at hand. A small outlay will equip a personal laboratory moderately well. Dependent upon the amount of work one expects to do, the larger pieces of apparatus may be simple or more capacious and costly. It is quite possible for one to dispense with an incubator provided he wishes to grow only a few organisms for clinical purposes. For instance, the culture-tube may be placed near a stove, where a temperature approximating that of the body will be maintained, or it may be carried in an inner pocket. Most organisms grow fairly well at room-temperature.

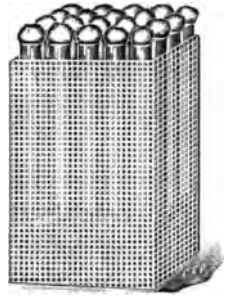

FIG. 31.—TEST-TUBE BASKET.

As a suggestion to him who wishes to fit up a laboratory for individual investigation on a limited scale, the following list of essentials is given. Much more may be added with advantage, and, on the other hand, some of the articles are not absolutely necessary, but will materially facilitate the work.

LIST OF NECESSITIES FOR BACTERIOLOGIC WORK.

Scales and metric weights up to 25 gm.
1 glass cylindric graduate 1000 c.c.
1 glass cylindric graduate 100 c.c.
1 glass funnel 170 mm. in diameter.
2 glass funnels 120 mm. in diameter.
2 pounds glass tubing for pipettes.

6 glass rods for stirring.
2 platinum needles.
100 filter-paper discs 24 mm. in diameter.
10 sheets bibulous paper.
1 gross test-tubes 150 × 15 mm.
3 Koch's flasks 1000 c.c.
6 Koch's flasks 500 c.c.
Cotton-wool for plugging tubes.
2 wire baskets.
6 Petri dishes 100 mm.
½ gross glass slips.
½ oz. glass covers No. 2 squares.
100 labels.
2 hollow-ground slips.
1 hot-air sterilizer, with thermostat, thermometer, and burner.
1 Arnold sterilizer or autoclave, with burner and tubing complete.
1 incubator, with thermostat, thermometer, burner, and tubing complete.
1 jar of beef extract.
Litmus paper (red and blue).
1 bottle peptone (Witte's).
6 fermentation tubes.
1 Cornet forceps.
1 cover-glass forceps.
2 test-tube brushes.

DRY STAINS AND CHEMICALS.

30 gm. methylene-blue.
30 gm. gentian-violet.
30 gm. eosin-yellow.
30 gm. fuchsin.
10 gm. Bismarck-brown.

50 gm. carbolic acid crystals.
10 gm. iodin crystals.
20 gm. potassium iodid.
50 c.c. glacial acetic acid.
30 c.c. Canada balsam.
50. gm. potassium hydrate.
50 gm. pyrogallic acid.
10 gm. sodium hydrate.
10 gm. litmus.

PREPARATION OF CULTURE-MEDIA.

In making culture-media absolute cleanliness must be observed throughout the entire procedure. Every possible source of contamination must be avoided when once the medium has been made. Each time that it is exposed to any infection it must be sterilized. Never allow that portion of the cotton plug that enters the Koch flask (Fig. 35) to touch any object that will contaminate it. If it should be thoughtlessly laid upon the table while the medium is being poured from the flask, it should be replaced in the flask and the whole sterilized at once.

There are many ways in which contamination of media may take place, and one can only learn by experience to be bacteriologically "clean" in his manipulations.

The following directions are made simple and explicit for the sake of the beginner, each step being laid down *seriatim*.

Bouillon consists of:

Beef extract,	3 gm.
Peptone (Witte's),	10 gm.
Sodium chlor.,	5 gm.
Water,	1000 c.c.

Put the water in a clean agateware saucepan and heat by means of a radial burner or on the stove. The solid constituents should be weighed out upon a piece of paper and stirred with a glass rod into the water after it has reached the boiling-point. The paper should be thrown into the water, in order that any beef that clings to it may not be lost, and as soon as the beef has dissolved the paper is fished out with the stirring-rod.

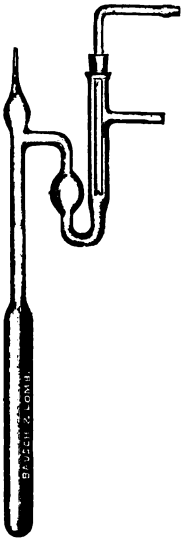

FIG. 32.—DR. DUNHAM'S THERMOREGULATOR.

FIG. 33.—FERMENTATION TUBE.

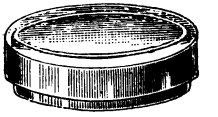

FIG. 34.—PETRI DISH.

The mixture is gently boiled for five minutes. It must now be made neutral or *very slightly* alkaline. For this purpose two solutions are kept on hand—a 5 per cent. solution of sodium hydrate and a 5 per cent. solution of hydrochloric acid.

One strip of red and one strip of blue litmus paper are

placed side by side on a piece of clean filter-paper and with the glass stirring-rod a drop of bouillon is put on each of the strips. If the bouillon is found to be acid, as is usually the case, a few drops of sodium hydrate are added to the mixture, it is vigorously stirred, and again tested, sufficient soda solution being added to make the bouillon neutral or, better, very slightly alkaline, so that it will turn the red litmus paper a faint blue color. If this stage is inadvertently passed, a few drops of the hydrochloric acid solution are used. Should the bouillon when originally tested be alkaline, it may be necessary to neutralize with the acid solution.

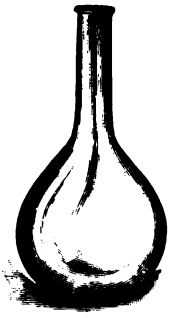

FIG. 35.—KOCH FLASK.

The variation of the reaction of the bouillon depends upon the meat extract. At times we may obtain an extract that will be quite acid, and again one will make a bouillon of marked alkalinity.

The bouillon must be allowed to cool in order that the meat salts may be precipitated and be filtered through filter-paper, folded in the usual way and previously wetted with water and pressed into the stem of the funnel. Sufficient water must be added to bring the mixture to the original quantity of 1000 c.c.

If it is desired to preserve the medium in bulk, it must now be placed in a Koch's flask, the mouth of the flask closed with a large and light plug of cotton-wool, and the whole sterilized. The bouillon may also be drawn into test-tubes and sterilized for immediate use.

Bouillon should be of a clear amber color.

Frequently bouillon is modified by the addition of one of the sugars, so that we may have a glucose bouillon, lactose bouillon, and sacchrose bouillon. In each case

1 per cent. of sugar is added to the formula for plain bouillon.

Glycerin bouillon consists of plain bouillon to which has been added, *after filtration*, 5 to 8 per cent. of a good quality of glycerin.

Nutrient Gelatin.

Beef extract,	3 gm
Peptone (Witte's),	10 gm.
Sodium chlorid,	5 gm.
Gelatin (gold label),	100 gm.
Water,	1000 c.c.

It will be seen that a gelatin medium is simply bouillon with the addition of gelatin, which solidifies when the medium is cooled.

The first steps in making gelatin are identical with bouillon preparation, except that the mixture must be subjected to the minimum amount of heat. Prolonged boiling of gelatin diminishes its solidifying qualities.

When the gelatin is dissolved, the mixture is neutralized and allowed to cool to 60° C., and to it are added two fresh eggs beaten up in 250 c.c. of water. Gentle heat is added so that the mixture will slowly come to the boiling-point of 100° C. *It should not be stirred at all* during the process, neither should it be allowed to boil vigorously, but only to simmer. As the temperature rises it will be noticed that the albumen of the egg gradually coagulates in fine flakes which finally rise to the surface and form a thick scum.

When by gently breaking the scum with a glass stirring-rod it is seen that the medium is a clear amber color, the vessel is to be removed from the source of heat and the medium is ready for filtering. The egg is added to clear the solution, which would otherwise be very cloudy.

If after filtering and cooling the medium is still cloudy, it may be cleared by adding another egg and proceeding as in the first instance. It is of importance that the medium be perfectly clear and free from deposit, otherwise we may make wrong deductions from cultures made on it.

The melting-point of gelatin is 25° C. and it becomes solid at 10° C. As the incubator temperature is 37° C. gelatin media cannot be subjected to incubation.

In summer it is frequently necessary to keep the gelatin tubes in an ice-chest. Using more gelatin, up to 1.5 per cent., will give a firmer medium. The fact that in the process of growth some bacteria liquefy gelatin while others do so in less degree or not at all, renders it a valuable agent for differentiating organisms.

Glucose gelatin is made by adding 1 per cent. of glucose before sterilization, and may be used for growing anaerobes.

Filtering.—While with bouillon it makes little difference what kind of filter-paper is used, with solid media that made by Schleicher & Schnell and water-marked No. 595 is far preferable to any other the author has used. With this particular brand agar may be filtered without the aid of the hot-water apparatus and in a few moments. The filter-paper should in all cases be properly folded and a ribbed funnel used, as this will greatly facilitate filtering. When the paper has been folded and put in the funnel, it should be moistened with a small quantity of hot water and gently pressed downward so that the end of the filter-paper enters the stem of the funnel a short distance. This supports the point of the paper, which is weakened by folding, and prevents its rupture when the weight of the medium comes upon it.

It is well with this, and other media where the egg has

been used, to strain it through a fine wire collander or through a piece of cheese-cloth held over the filter.

Either of these methods rids the fluid of the larger coagula of egg and renders the filtration quicker.

Agar-agar.

Beef extract,	3 gm.
Peptone,	10 gm
Sodium chlorid,	5 gm.
Agar-agar,	10 gm.
Water,	1000 c.c.

Agar is the product of an eastern seaweed and is added to the bouillon as a solidifying agent, it having no nutrient properties of itself. Agar comes in the form of shreds and also in square sticks. The stick is preferable because it is easier melted.

It is difficult to melt agar, and whatever the form, it should be cut into small pieces before being added to the liquid, and the whole should be boiled until the agar is thoroughly dissolved, water being added when necessary to compensate for evaporation.

A mark may be made on the side of the vessel to indicate the line reached at 1000 c.c. of fluid. Scum that forms should be skimmed off. When the agar is melted, the medium is neutralized, cooled to 60° C., and two eggs stirred into 250 c.c. of water and added. Careful heating and coagulation of the albumen is proceeded with as in making gelatin.

Agar must be filtered hot into a Koch flask. If Schleicher & Schnell's filter-paper is used, no difficulty should be experienced. The coagulated egg is to be removed by straining through a fine collander or through a piece of cheese-cloth. It is well to pour only a part of the medium into the filter at a time, the remainder being kept

hot. If the agar becomes cool and solidifies in the filter, it must be melted and filtered through a fresh paper.

The agar may be modified by the addition of 1 per cent. of glucose, lactose, or saccharose, or 5 to 8 per cent. of glycerin. These substances should be added to the medium *after* filtration, but *before* sterilization.

Litmus milk is useful in determining the reaction of bacteria during growth and their power to coagulate the medium. It is easy to make and is a good culture-medium.

FIG. 36.

The milk should be fresh and freed from most of its cream, by the centrifugal method or by skimming after it has been set aside for a time in the refrigerator. Milk is generally alkaline, but the reaction should be tested and corrected if necessary. To the skimmed milk is added a sufficient quantity of an aqueous solution of litmus to impart a distinct blue color.

The milk is then put in tubes and sterilized. The heat may cause the color to fade, but this will return in a few hours; otherwise more litmus must be added and the tubes again sterilized.

Potatoes make a useful medium for special purposes. Old Irish potatoes are to be well scrubbed under running water with a stiff brush and peeled, the eyes taken out, and cylinders of proper size to fit a large test-tube cut out with a tin apple-corer. These cylinders are then cut in a slanting direction into two pieces (Fig. 36), each of which will present a sloping surface for inoculation. The plugs are washed overnight in clean running water, and then, butt-end

down, placed in tubes and sterilized when they are ready for use.

Trudeaux has recommended potato soaked in peptone bouillon as a medium for cultivation of the tubercle bacillus.

Potato tubes dry out and become discolored in a short time, so that the medium should be prepared frequently and not in large quantities.

DUNHAM'S MEDIUM OR PEPTONE SOLUTION.

Peptone,	10 gm.
Sodium chlorid,	5 gm.
Water,	1000 c.c.

The solids are dissolved in the boiling water; the mixture filtered and sterilized. The formation of indol during bacteriologic growth may be detected by the use of Dunham's medium. Tubes of the medium are inoculated and grown at incubation temperature for forty-eight hours, after which they are set aside in the dark for five days. At the end of this time add to the tube eight drops of strong sulphuric acid, shake, and warm over a flame. If no color develops, add 20 drops of a 5 per cent. solution of sodium nitrate, shake, and warm. If indol is present a rose color appears.

Blood-serum.—This is one of the most useful media. Blood may be obtained at the slaughter-house and should be collected in a large jar directly from the severed vessels of the neck. The first blood that flows should be allowed to escape, as it is contaminated with dirt and hair.

After a clot has formed in the jar it should be removed to the laboratory and placed in the ice-chest for twenty-four hours, or until the serum shall have separated from the clot. The clear, straw-colored fluid serum is now siphoned off, and test-tubes previously prepared are filled

one-third with it. The tubes are now sterilized and the serum coagulated in the special manner described under the proper head. It should be of a light fawn color when solidified.

Löffler's Blood-serum.—The serum is collected as above and mixed with glucose bouillon, in the proportion of three parts of serum to one part of bouillon. It is tubed and sterilized in the same way as plain blood-serum. Löffler's blood-serum is valuable for cultivating B. diphtheriæ, the tubercle bacillus, and organisms that do not grow well upon ordinary media.

Fresh blood-serum may be preserved in bulk until required by the addition of 5 per cent. chloroform. The flask must be well corked to prevent evaporation of the chloroform. When it is desired to use the serum, it is tubed and sterilized as above, the chloroform being volatilized and driven off in the process.

Human serum may be obtained in the form of hydrocele or pleuritic fluid or the effusion of ascites. It should be treated in the same way as blood-serum, and makes a good serum which is generally milky white.

Preparation of Test-tubes.

Test-tubes are generally used as containers for the media upon which bacteria are cultivated. As they come from the shops they are not clean and are continually being soiled by use. They are best cleaned by boiling in strong soap-water. If the tubes have been used for cultures, care must be taken not to allow their contents to come in contact with the furniture or instruments about the laboratory.

A suitable vessel for boiling is prepared, the cotton plugs are withdrawn from the tubes, and the tubes laid in rows in the vessel. The plugs are to be burned. The

vessel is filled with sufficient water to cover the tubes and a quantity of *turpentine* soap chipped up and added. The whole is boiled for about an hour. This will kill the organisms present and prevent infection, and the media in the tubes will be softened if not boiled out. The vessel is put under the spigot and cold water run into it until the contents are cool enough to allow the tubes to be removed by hand. The tubes are thoroughly rinsed in cold water, inverted in a wire basket, and set aside to dry. Should it be necessary, the test-tube brush may be used for cleaning tubes in which the medium has not been boiled free.

Plugging Tubes.—When the tubes are dry, they are to be plugged with cotton-wool. The plug should be about two inches long, and fit tight enough to support the weight of the tube. Plugs are best made by putting a piece of cotton of suitable size upon the upper surface of the closed fist and with the index-finger of the other hand forcing the cotton through the ring formed by the first finger and thumb. The cotton is given a squeeze, removed, and placed in the neck of the tube with a twisting motion, as in driving a screw. In subsequent removal and insertion of the plug this twisting to the right should always be practised, as it keeps the cotton tight and facilitates the work. Absorbent cotton is unsuitable for plugs. Ordinary cotton batting is best for the purpose.

Sterilizing Tubes and Petri Dishes.—The plugged test-tubes are now placed in the hot-air sterilizer or in an oven and subjected for an hour to 150° C. The purpose of this procedure is rather to set the plugs than to sterilize the tubes. The heat should have been sufficient to turn the cotton brown without scorching it.

The plugs do not have the former tendency to unroll and spread when taken from the tubes. Petri dishes are

sterilized by submitting them, *covers on*, to 150° C. for one hour on two successive days.

Filling the Tubes.—The tubes are most conveniently

FIG. 37.—FILLING TEST-TUBES.

filled by means of a glass funnel supported on an iron stand and having on the stem a piece of soft rubber controlled by a pinch-cock and provided with a short glass nozle for

insertion into the tube. Fig. 37 illustrates the method perfectly. Of course, gelatin and agar must be melted

FIG. 38.—AUTOCLAVE

before tubing if they have previously been allowed to solidify. Care should be taken to prevent any of the

medium coming in contact with the neck of the tube, as this will cause the plug to stick and give trouble.

As the tubes are filled they are set aside in a wire basket preparatory to sterilization. For ordinary purposes the tube should contain about one inch of medium.

Sterilization of media is necessary in order to kill any spores or organisms present. This may be accomplished

FIG. 39.—ARNOLD STEAM STERILIZER.

either by dry or moist heat or by chemicals. The latter method is not applicable to media.

Dry heat is not nearly so effective as moist heat, and is used comparatively little. Media in bulk may be sterilized by boiling for half an hour or, better, by the use of the autoclave (Fig. 38), which is designed to give a temperature of 110° C. under the pressure of one atmosphere—15 pounds to the square inch. Unfortunately, the apparatus

is so expensive as to be out of the reach of the ordinary worker.

In the autoclave media may be thoroughly sterilized in a single subjection to steam heat at the above pressure for ten minutes. Gelatin should be autoclaved for five minutes only.

If an autoclave is not at hand, sterilization may be accomplished by means of the Arnold sterilizer (Fig. 39). With this instrument we must resort to fractional sterilization; that is, the medium must be sterilized for at least fifteen minutes upon each of three successive days.

FIG 40.

To be sure that the tubes are sterile, it is well to put them in the incubator for twenty-four hours, and if no growth occurs they may be safely used. After the sterilization of agar some tubes should be inclined and allowed to cool in that position. This gives a sloping, even surface for inoculation, and is known as a "slant" tube. The tubes which cool in the upright position are to be used for stab cultures (Fig. 40).

Blood-serum tubes are to be *sterilized* in the inclined position. They should be subjected for half an hour upon four successive days to a temperature of 80° C. Upon the fifth the temperature is to be gradually raised to 95° C. and kept at this for half an hour.

The media being now solidified, may as a precautionary measure be sterilized in the autoclave or steam sterilizer. A special apparatus for coagulating blood is shown in Fig. 39.

The Arnold sterilizer may be used, as by leaving the outer jacket off a sufficient heat to coagulate the serum

FIG. .41—SERUM SOLIDIFYING APPARATUS.

FIG. 42.—PHYSICIAN'S HOT-AIR STERILIZER.

is produced, while it is not brought to a boil. If the heat

be too high before the serum is solid, it will produce bubbles throughout the medium.

Hydrocele, pleural and ascitic fluids are to be treated according to the methods laid down for blood-serum. Potatoes and milk require the same treatment for sterilization as do bouillon and agar.

If no better means of sterilization are available, it may be accomplished (except with potato and blood-serum)

FIG. 43.—HOW TO INOCULATE CULTURE-TUBES.

by boiling the medium in a test-tube by the aid of a bunsen burner or spirit-lamp.

Inoculation of Tubes.

For the purpose of transferring bacteria into and from test-tubes the platinum needle is generally used. This consists of a piece of platinum wire about three inches in length set in the end of a glass rod eight inches long.

Two needles should be kept on hand, one being bent into a small loop at the end and the other straight. The former is for picking up large masses of material, the latter serves for making **stab cultures.**

How to Inoculate a Tube.

Inoculation of media contained in a test-tube should be done in a routine manner. If one in the beginning forms correct laboratory habits, it will aid him in all his future work. If inoculation is to be made from one tube containing a culture to one containing fresh media, the following method should be followed:

Take the culture-tube, pull out the cotton plug, hold it

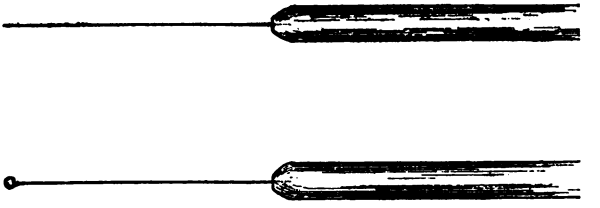

FIG. 44.—PLATINUM NEEDLE AND LOOP.

carefully by the top part, and do not allow that portion which fits into the tube to touch the fingers or any other object.

Keep the tube inclined, so that the dust of the air will not fall into it, and pass the *neck* of the tube several times rapidly through the bunsen flame or spirit-lamp flame; replace the plug and treat the second tube in like manner. If when the plug is removed from the tube some of the cotton sticks to the neck, it may be burned out by holding the tube in a horizontal position and bringing the mouth of the tube quickly in contact with the bunsen flame.

The above procedure, called "**flaming,**" is for the purpose of preventing possible contamination by bacteria which may have gained access to the neck of the tube and which might fall into it in subsequent manipulations.

Both tubes are held in the left hand, palm uppermost, in a slanting position, the tube from which the culture is to be made being next the thumb.

The cotton plug is taken from this one and its upper extremity carefully placed between the index and middle fingers; the cotton plug from the second tube is placed in like manner between the ring and little fingers. The platinum needle is grasped in the free right hand and sterilized in the bunsen flame by heating its entire length a cherry-red; the glass handle is passed several times through the flame so that the portion that will enter the tube will be thoroughly sterile. It is well to introduce the needle first into the fresh tube and cool it in the medium; it is then inserted in the other tube, touched to the growth of the culture, brought out without touching the sides of the tube, and introduced with the same care into the fresh tube.

If the medium is fluid, the needle should be shaken about in it; if solid and slanted, the needle-point should be brought in contact with the surface of the bottom of the slant and drawn along the middle of the surface until the upper limit is reached.

One inoculation is sufficient. If the medium has been solidified with the tube vertical, the needle is thrust once deeply into the center of the plane surface. Before putting the needle down, it must *always* be sterilized in the flame.

The cotton plugs are now replaced in their respective tubes.

Labeling.—The tube which has been inoculated must now be labeled with the name of the bacteria, if known,

date of inoculation, and, if there are several workers in the laboratory, with the name of the person making the culture. The date is indicated as in Fig. 41, the day and year being written in Arabic numerals, the month in Roman numerals. Gummed labels can be purchased for this purpose.

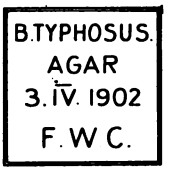

FIG. 45.

Incubation.—We have seen that pathogenic organisms grow best at the temperature of the body. If the inoculation has been made upon any medium except gelatin,

FIG. 46.—LABORATORY INCUBATOR.

the tube should now be put into the incubator for from twenty-four to forty-eight hours, or longer in the case of a

few organisms. An incubator for individual work may be purchased at a small outlay, or improvised, if one is ingenious, from two tin boxes.

The temperature should be 37° C., or 98.6° F., and must be regulated by a thermostat of some description. Dunham's thermostat is very satisfactory. Most organisms grow well at room-temperature.

It is always better to keep cultures in the dark, as they grow better.

Plate Cultures.

For the purpose of separating organisms, cultures are made in Petri dishes (Fig. 34). These consist of shallow flat-bottomed dishes with loose glass covers, and may be obtained of various sizes, those having a diameter of 100 mm. being preferable.

If a culture is supposed to contain more than one variety of organism, we may proceed to make plate cultures as follows: Three tubes of agar are placed in a water-bath at boiling temperature until they are melted; cold water is added until the temperature of the bath reaches 40° C., at which temperature the organisms to be inoculated will not be killed and the agar will still be fluid.

The tubes should be labeled one, two, and three, and three sterile Petri dishes are set in a row on the table. A tube of sterile bouillon is now inoculated with one loopful of culture we wish to plate. The bouillon should be well agitated with the platinum needle, the needle again sterilized, and agar tube No. 1 inoculated with three loopfuls from the bouillon tube.

The needle is sterilized and agar tube No. 2 inoculated with three loopfuls from agar tube No. 1. Agar tube No. 3 is inoculated with three loopfuls from agar tube No. 2. Beginning with agar tube No. 1 each agar tube is emptied

into a Petri dish, the dish labeled with the number of the tube and date of inoculation, and when the agar has solidified put into the incubator. *Before pouring the melted agar into the Petri dish the neck of the test-tube must several*

FIG. 47.—PLATE CULTURE.

times be passed rapidly through the flame of the bunsen in order to sterilize it.

The bouillon tube is used only for the purpose of diluting the original culture. After twenty-four hours' incu-

bation the plates should be examined. If the growth has taken place, it will probably be found that plate No. 1 contains a great many "colonies," which may be so numerous as to be fused. Plate No. 2 contains fewer areas of growth, while in plate No. 3 not more than a half dozen "colonies" are found.

The colonies may now be studied, without removing the cover of the dish, with the low power of the microscope or with a hand lens. Stained preparations should also be made. Dissimilar colonies should be "fished" with a sterile platinum needle and planted in fresh culture-tubes. By this method we may obtain in pure culture any organism present.

Only a minute quantity of the original culture should be taken, else the growth, even in the third plate, may be so dense as to thwart the purpose in view.

Method of Obtaining Cultures from Living Animals.

Blood.—It is, of course, necessary to secure the material free from outside contamination. In a human being a vein of the arm is generally selected as the part from which the blood can best be obtained. The region of operation must be cleansed with the same care that would be taken in surgical procedure, scrubbing with soap and water, shaving, and the final use of ether and alcohol being necessary.

With a sterile syringe about 5 c.c. of blood is drawn from the vein and transferred to six test-tubes containing various media. It is well to use two tubes of bouillon, two slant agar, and two blood-serum tubes. The blood should be smeared freely over the surface of the slant tubes by tilting them, or, if necessary, with the sterile platinum loop. They are then labeled and incubated.

Urine.—The external genitals having been cleansed,

the urine may be drawn into a sterile vessel by means of a sterile catheter. Inoculation of media should be made as soon afterward as possible, as otherwise we may be led into error as to the relative number of organisms present, and it is also quite possible that in spite of precautions contamination may have occurred and saprophytes may grow and obscure the pathogenic organisms for which we search. Of course, there must be no antiseptic added to the urine. It is well to examine some of the urine microscopically when procured, or as soon after as possible.

It should be remembered that it is scarcely possible to cultivate tubercle bacilli from urine, though they may be present, and that their detection in urine is better accomplished by other methods. Bouillon, slant agar, and plate cultures should be made.

Feces should be collected in a sterile vessel and bouillon and plate inoculations with small bits of the material should be made as soon as possible. If the feces are solid, they should be torn apart with sterile forceps and inoculations made with the material obtained from the center of the mass, as this will be free from possible contamination.

The Throat.—It is frequently desirable to obtain cultures from the throat in suspected diphtheria and in other conditions. The plan followed by the various Boards of Health is a good one.

A swab is made from a small piece of absorbent cotton wound on a stout wire about 15 cm. long. The swab is put into a clean test-tube, which is plugged as for media and sterilized by dry heat (150° C. for one hour, or, better, by the usual method of steam sterilization). This sterile swab, incased in the tube, may be carried to the bedside. The cotton end should be rubbed over the suspected area

in the throat, care being taken to touch no portion of the buccal cavity, and then drawn several times over the surfaces of two slant blood-serum tubes. The swab should also be used for smearing two glass slips with the suspected material, as it may be possible to make an immediate diagnosis from these. When the inoculation is finished, the swab is put back into the original tube, taken to the laboratory, and carefully sterilized.

The inoculated tubes are to be incubated.

Sputum.—It is very difficult to obtain sputa uncontaminated by mouth bacteria.

The mouth should be cleansed with a tooth-brush and some antiseptic, such as Listerine, and the sputa received in a sterile vessel. Tubes should be immediately inoculated. It is seldom necessary in clinical work to make cultures from sputa; direct microscopic examination generally furnishes quite as much or more information. If such is not the case, we may inoculate an animal and obtain the desired result.

Pus from abscess cavities may be smeared upon slant agar tubes or planted in bouillon by the aid of the sterile swab or the platinum loop. It must be remembered that it is scarcely possible to grow the tubercle bacillus directly from an abscess, even if it be tubercular in origin, and that a certain conclusion can be arrived at only after animal inoculation.

Autopsy Methods.—Bacteriologic investigation should be made in all cases coming to autopsy. Inoculation of bouillon and of glucose agar tubes should be the rule. The agar must be melted in a water-bath at the boiling-point; the water is then cooled by adding cold water until the temperature is 40° C., at which the bath may be kept by aid of a bunsen burner or spirit-lamp.

The agar at this temperature will remain fluid, and such

a degree of heat will not affect any organisms that may be planted in it.

Cultures should be made from the pericardium, heart's blood, lungs, spleen, kidney, and liver, and from such other situations as the character of the case may render of interest. The inoculation should be made with the platinum loop of large size, as we wish to transfer a considerable amount of material to the tube, for the reason that the organisms may be present in small numbers. Three loopfuls of material should be taken for each tube.

The instruments necessary are one small scalpel, one case-knife, one thumb-forceps, and the platinum loop.

Great care must be exercised not to contaminate the cultures from outside sources. The tubes must be flamed and held as already described, the needle carefully sterilized, and the site from which the inoculation is to be made prepared as follows:

It is probable that the first inoculation will be made from the pericardial fluid. The end of the case-knife is heated to a cherry-red and laid upon the exposed surface of the pericardium. This will sear and sterilize the area touched. The small scalpel is sterilized in the flame and plunged through the center of the sterilized spot, the pericardium being steadied by the aid of the forceps, which should not touch the sterile area unless their points have also been flamed. The sterile platinum loop is now introduced through the opening and one tube of bouillon and one tube of glucose agar inoculated with three loopfuls of the pericardial fluid. The neck of the agar tube is at once flamed and its contents poured into a sterile Petri dish the cover of which is not removed but simply raised on one side sufficiently for the purpose.

The Petri dish is to be labeled and set on a level place, so that the medium, when cool, will be of a uniform

depth. The surface of each organ must be sterilized by the hot knife and inoculation made from it in like manner.

The immediate plating of the agar serves to simplify future work and gives an approximate idea of the number of organisms present. Glucose agar is used in the place of plain agar in order that gas-producers may at once be detected.

In cases where the presence of an anaerobe is suspected, as in tetanus, one must follow the technique necessary for the growth of anaerobes.

In addition to cultures, film preparations on slips or cover-glasses should be made of the blood, pulp of organs, or pus, and excessive serous accumulations, if such be present. Portions of tissue should also be fixed in synthetic alcohol and preserved for future study.

Methods of Cultivating Anaerobes.

Anaerobes must be grown in an atmosphere other than air. Hydrogen is frequently used, but an apparatus for generating it is not available except in well-equipped laboratories. Two simple methods will be outlined.

No. 1.—Select test-tube at least 15 mm. in length and fill it three-fourths with agar or gelatin. Boil the medium for a few minutes either in a water-bath or over the bunsen flame to drive off as much oxygen as possible. Cool the tube quickly in cold water until the medium is solid. With a long straight platinum needle make a deep stab inoculation extending almost to the bottom of the medium. On top of the medium pour a thin layer of melted paraffin, which has been boiled to sterilize it, plug the tube, and cover with a rubber cap or seal with melted paraffin or wax to exclude the air. If the medium is agar, it should be incubated. Growth will occur along the lower two-thirds of the stab.

No. 2 (*Buchner's Method*).—Inoculate tubes of media in the ordinary way, replacing the plugs. Select a glass fruit-jar in which the tubes may be stood. In the bottom of the jar put a layer of sand about ½ inch in thickness to guard against breaking the tubes, add two grams of pyrogallic acid, put the tubes in place in the jar, and pour in 200 c.c. of a 1 per cent. solution of potassium hydrate. The cover of the jar is to be immediately screwed down, the rubber ring having been previously coated with vaselin to render it air-tight. The jar may now be placed in the incubator for forty-eight hours, when the tubes may be taken out and studied.

As many anaerobes are gas-producers, it is well to use one or more tubes of glucose agar.

Methods for Determining Gas Production.

To determine the amount of gas produced during bacteriologic growth, the fermentation tubes designed by Dr. Einhorn are in general use (Fig. 33). They are to be plugged and sterilized as with ordinary tubes. The vertical arm of the tube should be filled to the neck with bouillon to which 1 per cent. of one of the sugars (glucose, lactose, or saccharose) has been added. After sterilization the medium is inoculated with the gas-forming organism and the result noted. One should observe the time when gas begins to collect in the top of the tube and measure the daily accumulation.

Hanging-drop Culture.

The development of bacteria may be studied under the microscope by inoculating a drop of culture-medium put on the center of a cover-glass and inverted over a hollow ground glass slip. The margin of the cover-glass should be sealed with vaselin to make the chamber air-tight.

Bacteria from cultures grown on solid media may be examined in the hanging-drop by putting a drop of water on a cover-glass and inoculating it with a portion of the growth. A loopful from the bouillon cultures may be utilized in the same way without dilution. The edges of the cover-glass should in each case be sealed with vaselin to prevent evaporation.

The hanging-drop is utilized to determine the motility of an organism or to study its development.

STAINING METHODS.

While a great variety of methods have been devised for staining bacteria, only those will be mentioned that are in common use. Most organisms stain well with the anilin dyes, and stains consist largely of these. Some bacteria require special staining methods, and with a few a mordant must be used in order to stain them at all.

Making the "Film."—In order that we may properly see the organisms after they are stained, it is necessary that they be spread in a thin even layer, so that they will lie side by side and not in a jumbled mass.

With a clean cloth polish a cover-glass until it shows no dirt on its surface. Holding the cover-glass in the Cornet forceps, pass it a number of times through the bunsen flame, to burn off any grease that may still be upon its surface. This is called flaming, and is essential to success.

With the platinum loop put a drop of clean water in the center of a cover-glass, sterilize the loop, and with it take a minute quantity of the culture and transfer it to the

drop of water. The drop should not be milky with bacteria, and it is generally necessary to touch it only once with the impregnated loop. With the straight part of

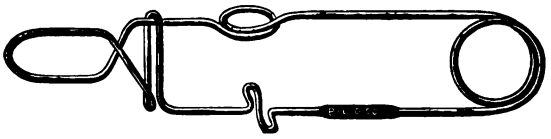

FIG. 48.—DR. STEWART'S COVER-GLASS STAINING FORCEPS.

the needle laid flat upon the center of the glass the drop is quickly spread in either direction.

If the glass has been thoroughly cleansed, there will be no clumping of the water and an even smear will result.

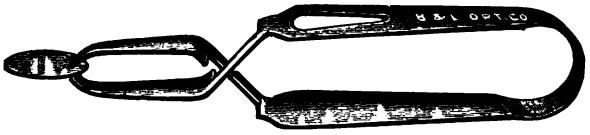

FIG. 49.—CORNET'S COVER-GLASS STAINING FORCEPS.

As soon as the film has dried pass the cover-glass three times through the bunsen flame to "fix" the bacteria and prevent them from being washed off in the further process

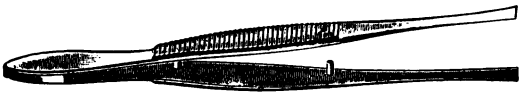

FIG. 50.—COVER-GLASS FORCEPS.

of manipulation. The film may now be stained according to the method selected.

Films prepared in the above way will keep for a long time and may be stained at will.

For clinical purposes, smears of the fluids of the body,

urine, serum, etc., may be made in like manner. Smears may be made upon the glass slip instead of upon the cover-glass, and this is often of advantage, as the examination of the stained film may be made without the use of the cover-glass, a drop of cedar oil being put directly on the film. If it is decided to make a permanent mount of the specimen, a drop of Canada balsam and cover-glass are added.

Methylene-blue is used extensively as a general stain and as an ingredient in several important special stains, and is perhaps the most useful stain at one's disposal, for it acts quickly and there is little danger of overstaining, which is not the case with some other of the anilin dyes.

The film prepared as indicated is stained for one minute with a saturated solution of methylene-blue.

The better way to apply the stain is put a few drops upon the cover-glass as it is held in the Cornet forceps. The preparation is then washed under the tap, blotted with bibulous paper, and thoroughly dried by passing it rapidly through the bunsen flame a few times.

A drop of balsam is put in the center of a glass slip and the cover-glass, "butter" side down, is laid upon it. The spread of the balsam may be facilitated by gentle heat, and the cover-glass should always be lightly pressed down with the end of some clean blunt instrument.

The object is now ready for study. If a $\frac{1}{12}$ oil immersion lens is used, one should afterward not endeavor to clean the oil from the cover-glass further than to blot it with bibulous paper.

When the balsam has been hardened by time or baking, the oil can be removed with a cloth wet with xylol.

RECAPITULATION.

1. Methylene-blue (not methyl blue) 1 per cent. solution, one minute.
2. Wash well.
3. Blot with bibulous paper.
4. Dry by passing through bunsen flame.
5. Mount in balsam.

If we wish to ascertain whether the bacteria have been sufficiently stained, the cover-glass may after step No. 2 be mounted in a drop of water, the surface blotted, and an examination made with the dry lens. Preparations mounted in water always look paler than the same preparation would if mounted in Canada balsam.

If we are satisfied with the result, the cover-glass may be slid from the slip, dried, and mounted in balsam. If desirable, the organisms may again be submitted to the action of the stain.

Ziehler's Carbol-fuchsin.—

Fuchsin (not acid fuchsin),	1 gm
Carbolic acid, pure,	5 c.c.
Alcohol,	10 c.c.
Water,	100 c.c.

This is a good general stain and acts very quickly, coloring the bacteria a brilliant red. It is also used in staining tubercle bacilli, special technique for which will be given under the proper head. The general technique is the same as that given for methylene-blue.

Gentian-violet is a rapid stain and is generally used with anilin oil. The following solution keeps for several months and is very useful:

Anilin-water-Gentian-violet.—

Anilin oil,	1 c.c.
Water,	100 c.c.

Shake the mixture until the oil is well distributed and filter through paper. To 80 c.c. of the above add 20 c.c. of a saturated alcoholic solution of gentian-violet. Mix and filter. The solution should be refiltered once a month. The process of staining is the same as with methylene-blue except that the stain acts almost instantly, and if allowed to remain in contact with the bacteria for more than a few seconds will overstain them.

FIG. 51.—BOTTLE FOR STAINING FLUID.

Gram's method is of particular utility because some of the most important pathogenic bacteria are stained by it, while others similar in morphology give up their color.

It is thus a valuable diagnostic agent. To obtain reliable results one must be very accurate in his technique, and the steps of the method must be followed exactly, the watch being used in timing.

1. Stain in *freshly made* anilin gentian-violet one and a half minutes.
2. Wash in water.
3. Gram's iodin solution one and a half minutes.
4. Wash in water.
5. Alcohol (95 per cent.) three minutes.
6. Wash in water.
7. Dry.
8. Mount in balsam.

Gram's iodin solution (step No. 3.) consists of:

Iodin (resublimated),	1 gm.
Potassium iodid,	2 gm.
Water,	300 c.c

Organisms that retain the stain are deep blue-black. Any organisms that are pale and ill defined may be considered decolorized.

Do not use a culture of less than ten hours' growth or one older than twenty-four hours or results will be unreliable.

A contrast stain which will restain the organisms which have been decolorized by Gram's method is often used, the simplest is:

Bismarck-brown.—Saturated solution in equal parts of alcohol and water. Stain for three or four minutes. Bacteria stained by Gram will be a blue-black, those stained by Bismarck-brown a pale brown.

Pathogenic Bacteria Stained by Gram's Method:

Staphylococcus pyogenes aureus.
Staphylococcus pyogenes albus.
Staphylococcus epidermidis albus.
Streptococcus pyogenes.
Micrococcus tetragenus.
Micrococcus lanceolatus.
Bacillus diphtheriæ.
Bacillus tuberculosis.
Bacillus lepræ.
Bacillus anthrax.
Bacillus tetanus.
Bacillus aërogenes capsulatus.
Ray fungus or Actinomycosis.

Important Organisms not *Stained by Gram's Method:*

Gonococcus.
Diplococcus intracellularis meningitidis.
Bacillus of malignant edema.
Bacillus of glanders.
Bacillus of Friedländer.
Bacillus proteus.
Bacillus of influenza.

Bacillus of typhoid fever.
Bacillus coli communis.
Bacillus pyocyaneus.
Spirillum of Asiatic cholera.
Spirillum of relapsing fever.

Staining Spores.—Spores may best be shown by the following method:

Make a thin film, as usual, from a culture twenty-four hours old.

1. Ziehl's carbol-fuchsin.
2. Heat over the bunsen until stain steams; heat should be kept up for five minutes, more stain being added from time to time to compensate for evaporation.
3. Wash in running water for one-half minute.

FIG. 52.—FORMS OF BACILLI SHOWING SPORES.

4. Immerse in 1 per cent. aqueous solution of sulphuric acid one-half minute.
5. Wash in water.
6. Methylene-blue one minute.
7. Wash in water.
8. Blot, dry, and mount in balsam.

The sulphuric acid solution takes the color from the body of the bacteria, the spores retaining it. It is well to examine the preparation mounted in a drop of water after step No. 5.

If not sufficiently decolorized, it should be again put in the acid bath. Care must be taken not to allow it to re-

main there too long, as the spores may also give up their color. A perfect specimen should show the spores stained red and the bodies of the bacteria blue.

Staining of flagella is a difficult process, and it is often necessary to make repeated attempts before success is attained.

Löffler's method as modified by Bunge is as follows:

Mordant:

Saturated solution of alum, ...30 c.c.
Solution of liquor ferri sesquichlorid of the strength of 1 : 2 distilled water,.....10 c.c.

Very thin cover-glass films should be made of a culture about eighteen hours old. The mordant must be filtered immediately before it is used. The cover-glass, held in the Cornet forceps, is covered with a few drops of the solution and allowed to stand for five minutes. It is then gently warmed, washed in water, and dried.

Stain:

Mordant (as per above formula), ... 10 c.c
Saturated aqueous solution of fuchsin, ... 1 c.c

Must stand several days before it is ready for use.

1. Stain for one minute.
2. Wash.
3. Blot, dry, and mount in balsam.

Staining of Capsules.—The method devised by Welch is simple and generally effective.

1. Films made as usual.
2. Glacial acetic acid ten seconds.
3. Wash off thoroughly with carbol-fuchsin. (This is best accomplished by using several pipettes full of stain.)
4. Blot off stain and dry over bunsen for two seconds.
6. Dry and mount in balsam.

Animal inoculation plays an important part in laboratory work. The animals usually chosen are the guinea-pig, rabbit, rat, and mouse. These animals are selected on account of their susceptibility to various diseases and because their small size renders them more suitable. Susceptibility varies in different animals, the white mouse and rat being susceptible to diseases from which the brown variety is immune, and the same variation may occur between tame and wild animals of the same variety.

The guinea-pig is more susceptible to bacteriologic infection than any other animal, and is therefore most useful for laboratory experiment.

Inoculation is generally made with the hypodermic syringe, and may be subcutaneous, intraperitoneal, intravenous, or into the anterior chamber of the eye.

The syringe should have asbestos packing or be all metal, so that it may be sterilized by boiling prior to use, and the point chosen for inoculation should be shaved.

The ear vein is generally chosen for intravenous inoculation.

The eye should be anesthetized with a few drops of cocain 2 per cent. solution and irrigated with a saturated solution of boric acid.

If the material to be injected is already fluid, no difficulty is to be encountered; and if culture on a solid medium is to be used, an emulsion may be made with a sterile test-tube by rubbing up with the platinum loop some of the culture with sterile water.

Mice and rats are usually inoculated at the root of the tail; guinea-pigs and rabbits in the center of the abdomen.

It is sometimes desirable to inoculate with bits of tissue, as in the case of tubercle, actinomycosis, and glanders, in which case a small opening in the skin is made with sterile scissors. The subcutaneous tissue is loosened up

and the material inserted with a sterile platinum loop. The flap should be closed and sealed with collodion.

Inoculated animals should be kept in wire cages having a solid bottom and half solid sides, the door being on top. With such a cage the possibility of infecting neighboring objects, the floor, etc., is obviated and the cage may be sterilized by boiling.

After inoculation animals must be carefully fed and watched and their symptoms noted.

Autopsy should immediately follow the death of the animal, or if this is not possible, the carcass must be rolled in a bichlorid towel and placed in an ice-chest.

Autopsy on Animals.—The body should be thoroughly wet with a 1:1000 bichlorid solution. This keeps the hair out of the way and thoroughly disinfects it so that there is no danger of infection of culture-tubes and the air of the laboratory.

A convenient adjunct is a V-shaped metal-lined trough in which the animal may be stretched out by fastening the legs by string to nails in the edges of the trough. The trough should be disinfected by immersion in bichlorid solution after each autopsy. Instruments, scalpels, scissors, and forceps should be sterilized before and after use by boiling for five minutes in water containing 5 per cent. of washing soda. The hair may be shaved from the linea alba and the incision made through the skin, from the sternal notch to the pubes, and the skin reflected. It is well to take a fresh scalpel and to flame the one just used prior to opening the cavities of the body.

The organs and serous cavities, the joints, diaphragm, and muscles should be examined and smears of heart's blood and organ pulp made in the same way that one would proceed in an autopsy upon the human subject.

It is sometimes convenient to remove the viscera entire

and keep them on ice in sterile Petri dishes until wanted. Cultures in all cases should be made as soon as possible after death.

Bodies of animals dead of infectious diseases should be burned or buried deep in the earth.

Too much care cannot be exercised in precautionary measures in handling pathogenic organisms; many an observer has had cause to regret his carelessness, and several have paid the penalty with their lives.

How to Study Bacteria.

It is of the utmost importance that a systematic written record be kept of any series under observation. The following tabulation (modified from Williams) gives the various points to be considered:

1. Name.
2. Habitat or source.
3. Morphology, grouping.
4. Size.
5. Spore formation.
6. Motility, flagella.
7. Staining properties. (Behavior by Gram's method.)

GROWTH ON CULTURE-MEDIA.

8. Bouillon.
   - (*a*) Time of growth.
   - (*b*) Strongly cloudy, uniformly cloudy, etc.
   - (*c*) Presence of scum or pellicle on surface.
   - (*d*) Precipitate on sides or bottom of tube.
   - (*e*) Color of precipitate, whether amorphous, granular, or flocculent, diffusible, viscid.
9. Gelatin stab.
   - (*a*) Time of growth.

(*b*) Character of surface growth. Vigor, color, elevation, smooth, rough, translucent, moisture, luster.

(*c*) Character of deep growth. Extent and shape of liquefaction if present, vigor, color, translucency, smooth, granular, nodular radiations or outgrowths.

10. Gelatin plate, gross description.
    (*a*) Time of growth.
    (*b*) Surface colonies, color, number, round, irregular, elevation, size, diameter, extent of liquefaction.
    (*c*) Deep colonies. Color, opacity, shape, knobs or bosses, character of edges, liquefaction.
11. Agar slant.
    (*a*) Time of growth.
    (*b*) Vigor.
    (*c*) Color.
    (*d*) Elevation.
    (*e*) Translucency.
    (*f*) Moisture.
    (*g*) Smoothness.
    (*h*) Luster.
    (*i*) Condensation of water; color, cloudy, granular, viscid.
    (*j*) Colorization of medium.
    (*k*) Odor.
12. Glucose agar stab. Same as gelatin except no liquefaction occurs and presence or absence of gas-formation is noted and amount of same.
13. Agar plates. Same as gelatin. No liquefaction.
14. Potato. Same as plain agar, and:
    (*a*) Discoloration of medium.
    (*b*) Gas-formation.

15. Litmus milk.
    (*a*) Time of growth.
    (*b*) Reaction, red, blue, decolorized, and pale.
    (*c*) If coagulated, time of peptonization and liquefaction of clot. Peptonization may occur without previous perceptible coagulation.
    (*d*) Gas-formation.
    (*e*) Cream ring (ordinary white and bluish) acid, alkaline, or unaffected.
16. Blood-serum. Same as plain agar and:
    (*a*) Whether peptonized and liquefied or not.
17. Relation of growth to temperature.
18. Relation to oxygen.
19. Production of indol in Dunham's medium.
20. Pathogenesis, determined by animal inoculation, with liquid cultures of various ages.

Peptonization and liquefaction may occur from the action of a bacteriologic ferment akin to trypsin.

Until one becomes practically familiar with handling bacteria, it is well to work with non-pathogenic cultures.

These may easily be obtained by exposing an agar plate for a few moments to the air of the room, incubating, fishing, and transplanting the various colonies that develop. A few pure cultures of such harmless organisms as B. prodigiosus, Sarcina lutea, or B. subtilis may be obtained from some laboratory and utilized for the purpose of acquiring technique.

Pyogenic organisms are easily isolated from pus and may be found in the air and water. There is no dearth of material for study, for on every hand bacteria exist and invite investigation.

Bacteria of the Normal Human Body.

Bacteria are constantly present in all individuals and in a variety of situations. Some of these are saprophytes, some harmless parasites, and others are capable under conditions favorable to themselves of producing disease and even death.

The skin is constantly exposed to bacteriologic contamination and several pathogenic organisms are habitually found upon it.

The nasal passages, the mouth, and the pharynx contain a large number of species, most of which are incapable of doing harm, but others (as the pneumococcus, which is found in 20 per cent. of healthy throats) may become fatally pathogenic to the host.

In the smegma of the genitals is found an organism which very closely resembles in its morphology and staining reactions the tubercle bacillus. The smegma bacillus is non-pathogenic.

The gastro-intestinal tract normally contains many species of bacteria, perhaps the most important of which is B. coli communis. Here again is an organism that is generally productive of no ill results, but one that under circumstances favorable to its invasion of the system may give rise to a number of pathologic conditions of serious character. Thus it is that we constantly carry within ourselves the seeds of dissolution.

Koch's Law.—In order to prove that a given organism is the agent productive of a specific disease Koch considers that the following requirements must be met.

1. That the organism must always be microscopically demonstrated in the bodies of animals dying of the disease, and that it must be present in this disease and no other. That its location and numbers should be such as to satisfactorily explain the lesions present.

2. That the organism must be susceptible of isolation and cultivation upon artificial media.

3. That after repeated generations have been cultivated so that no suspicion of other agencies are present the organism injected into a susceptible animal will produce the disease in question.

PATHOGENIC BACTERIA.

The Pyogenic Bacteria.

The organisms which produce suppuration and allied conditions are known as **pyogenic** bacteria. Dependent upon varying conditions, the same organism may at one time produce a local inflammation, at another time a local suppurative process, and at another a general infection of the system or a rapidly spreading phlegmon of the most serious character. The factors determining the extent of growth are many, and include the fitness of the soil (animal body) and the virulence of the bacteria introduced. We know that animal tissues have a certain amount of resistive power to the growth of bacteria, and that the resistive power of the body is decreased by conditions that tend to lower the cellular vitality—traumatisms, starvation, fatigue, etc.

Septicemia is that condition caused by the entranc of a toxic or poisonous organism into the blood-stream and its multiplication there in considerable numbers.

Pyemia is that condition in which there occur multiple abscesses as a result of a deposit in the tissues of pyogenic organisms distributed by the blood- or lymph-currents.

Sapremia is the toxic condition resulting from the absorption of bacteriologic products.

While all bacteria under conditions peculiarly favorable thereto may produce suppuration, yet certain bacteria habitually manifest their growth in the animal body by bringing about inflammatory lesions, and to these the term **pyogenic** properly belongs.

Rosenbach, in 1884, first isolated the pus-producing organisms and grew them upon artificial culture-media. He described and named Staphylococcus pyogenes aureus, Staphylococcus pyogenes albus, Streptococcus pyogenes, and Micrococcus pyogenes tenuis. Since the above date many other organisms have been found in pus, among which the most important are Staphylococcus pyogenes citreus, Staphylococcus cereus albus, Staphylococcus cereus flavus, gonococcus, Diplococcus intracellularis meningitidis, Diplococcus lanceolatus, Micrococcus tetragenus, and the pneumobacillus, Bacillus coli communis, and Bacillus pyocyaneus. The staphylococci are prone to produce localized, sharply circumscribed, suppurative areas, while Streptococcus pyogenes generally causes inflammation which tends to spread rapidly by continuity of tissue, as in erysipelas.

Staphylococcus pyogenes aureus.

Habitat.—Air, dust, water, skin, buccal cavity, etc.

Pathogenesis.—Found in man and animals and many acute suppurative conditions of circumscribed form. Occurs sometimes in pure culture and again with other organisms.

It is the most common cause of boils and localized acute abscesses, but it may cause septicemia and pyemia. In the latter condition in man, the primary local infection is disseminated through the blood-stream and lymphatics.

Animals are not so susceptible as man and are affected by Staphylococcus pyogenes aureus according to the method of inoculation and the virulence of the culture.

Subcutaneous injections of pure culture generally produce no result or only a slight local inflammation. If the culture is a virulent one, a local abscess may result. In interperitoneal inoculations with virulent cultures the animals usually die in from two to nine days (Park). Intravenous inoculations usually produce a pyemia with suppurative foci in the kidneys, diaphragm, heart-muscle, and joints. The effect produced depends largely on the amount injected.

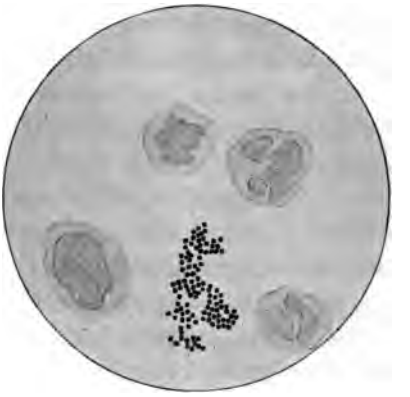

FIG. 53.—STAPHYLOCOCCUS PYOGENES AUREUS IN PUS.—(*Williams.*)

Morphology.—A spherococcus occurring in grape-like masses. The characteristic arrangement is best seen in paraffin sections of infected tissues stained by Gram's method.

Biology. — Aerobic, facultative anaerobic. Optimum temperature, 37° C.; maximum temperature, 44° C.; minimum temperature, 6° C.

Grows well at room-temperature. Recent moist cultures are killed in ten minutes by a heat of 58° C., while a temperature of 90° C. to 100° C. is required to kill the organism if it is dried (Williams).

Cultures in agar retain their vitality for a long time, perhaps a year or more.

According to Abbott, the resistive power to germicides

differs widely in organisms of the same culture, some being killed in five minutes by a 1 : 1000 solution of bichlorid of mercury, while others under the same circumstances retain their vitality for as long as thirty minutes.

Staining Reactions.—Stains by Gram and all the usual anilin dyes.

Bouillon.—The medium becomes uniformly turbid and later there is a thick deposit upon the bottom and sides of the tube. After a few days the deposit becomes yellowish.

Agar Slant.—At 37° C. generally a vigorous, opaque, white or pale brown growth within forty-eight hours, which later becomes bright yellow.

At times color production seems to be retarded by the particular specimen of agar upon which it is grown. Transplanting several times upon the same medium, or, better, upon potato, will restore the color-producing properties. The water of condensation will in a few days contain a thick, granular yellow mass of organisms.

Agar Stab.—Vigorous surface and deep growth, the surface assuming the characteristic color after a few days. The deep growth remains white because color production does not take place except in the presence of oxygen.

Gelatin Stab.—Vigorous growth at room-temperature along the whole needle track, but more pronounced at and near the surface.

Liquefaction of the medium begins at the top on the second or third day and a funnel-shaped depression rapidly forms and extends to the sides of the tube. Masses of streptococci settle to the bottom of the liquefied area and form the characteristic golden color from which the organism receives its name.

The gelatin is entirely liquefied in time.

Gelatin Plates.—At room-temperature colonies that

may be seen with the aid of the microscope develop in twenty-four hours. They present a coarsely granular pale brown appearance with a dark center and smooth edges. Later they become visible to the unaided eye as round, smooth, yellow colonies, which liquefy the gelatin and form little cup-like depressions.

Potato.—Vigorous, raised, moist golden yellow growth at room or incubator temperature.

Litmus Milk.—Rapid growth, with marked acidity, turning the milk red, and coagulation of the caseinogen.

The coagulum is peptonized and becomes liquid at a later period.

Staphylococcus pyogenes albus.

This organism is in all respects similar to the aureus except that the growth is white. Its pathogenicity is not so great as the aureus nor is the organism so common. There seems to be no reasonable doubt as to its being a distinct species, as all efforts to make it produce color have failed.

Staphylococcus pyogenes citreus.

Identical with S. aureus except that it produces a lemon color. Neither is it so common.

Staphylococcus epidermidis albus.

Found constantly in the human skin. It is not possible by present methods of cleansing the skin to rid it of this organism.

This accounts for its frequent presence in stitch abscesses, growth taking place on account of lowered vitality of the tissues from pressure of the suture.

Pathogenesis.—Very feeble; it never approaches that possessed by S. albus either in man or animals.

Morphology.—Identical with the aureus.

Cultural Characteristics.—Growth is slower and more feeble than S. albus.

Gelatin Stab.—Limited, raised, smooth, moist white surface growth. Along the needle track a scanty white growth. Liquefies the medium very slowly.

Litmus Milk.—Produces acid reaction and coagulates the medium, but much slower in action than S. albus. Welch considers this a distinct species on account of the differences in growth and pathogenicity. By some it is held to be a variety of S. pyogenes albus.

Staphylococcus cereus albus and **Staphylococcus cereus flavus** are comparatively rare. As the names imply, they produce a wax-like growth, the one white, the other yellow. Neither liquefies gelatin.

Streptococcus pyogenes.

Habitat.—Air, dust, pus, etc.

Pathogenesis.—Dependent largely upon the virulence of the culture. Streptococci which have been cultivated in the laboratory for several generations gradually lose their virulence, but it has been shown by Marmoret that the virulence can be restored and increased by cultivating alternately in a mixture of human blood-serum and bouillon and the body of a rabbit until a minute quantity inoculated into an animal will produce a septicemia, and death within a few hours. It is the most common cause of puerperal septicemia, and is now known to be identical with the streptococcus of erysipelas. It is frequently found in cellulitis and ulcerative endocarditis, and may produce in the throat a diphtheritic condition closely simulating true diphtheria.

Morphology.—A micrococcus about 1 μ in diameter forming chains, the members of which frequently lie in

pairs and are flattened on their contiguous surfaces. A variety forming short chains, Streptococcus brevis, and one forming long chains, Streptococcus longus, seem to be manifestations of the same organism.

Biology.—Aerobe and facultative anaerobe. Grows in all the usual media at room-temperature or 37° C. A much more delicate organism than the staphylococcus. It soon dies if not frequently transplanted.

Staining Reaction.—Stains by Gram's and by all the anilin dyes.

Bouillon.—Within thirty-six hours medium becomes diffusely cloudy or may contain small granules or flocculent masses. These sink to the bottom, forming a heavy deposit.

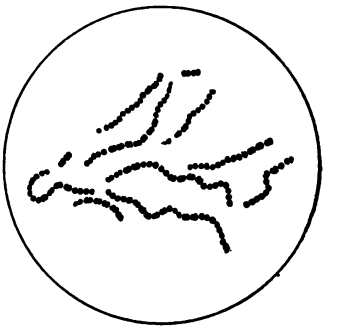

FIG. 54.—STREPTOCOCCUS PYOGENES.—(*Williams.*)

Agar Slant.—After thirty-six hours at 37° C. small white semitranslucent colonies along the needle track. The colonies average about 1 mm. in diameter and usually remain discrete.

Gelatin Stab.—No surface growth or liquefaction of the medium. Minute white colonies along needle track, more widely separated in lower part of stab.

Gelatin Plate.—Small white discs which do not liquefy the gelatin and which under the low power seem to be made of granules.

Litmus Milk.—Usually acid in reaction and coagulation.

Bacteriologic Diagnosis.—Streptococcus pyogenes may be frequently demonstrated in pus and in pseudo-diph-

theria or a mixed infection of pure diphtheria by cover-glass smears stained with methylene-blue or gentian-violet.

Cultures may be made by inoculating tubes with suspected material. In sections of tissue they may be stained by Gram's method (see p. 111). To isolate the organisms from a case of erysipelas a small piece of skin is taken from the erysipelatous patch, and after being crushed is added to a tube of melted gelatin at a low temperature. The tube is well shaken and the gelatin is poured into a Petri dish which is kept at a temperature of 20° C. Within forty-eight hours small colonies will begin to develop in the neighborhood of the particles of skin.

The above procedure must, of course, be under aseptic precautions.

Mice are very susceptible to inoculation with streptococci, and this furnishes a very ready means of diagnosis in septicemia caused by this organism. With a sterile hypodermic syringe draw from a vein 10 c.c. of blood. Half of this is injected into the peritoneal cavity of a mouse, and with the remainder a tube of bouillon is inoculated The mouse should die of septicemia, and its blood will contain the streptococci in large numbers (Petruschky).

The presence or absence of capsules to the organisms in the mouse blood so obtained furnishes a means of diagnosis between the streptococcus and pneumococcus (Park).

Micrococcus tetragenus.

Habitat.—Pus, sputum, lungs in tuberculosis, pus of empyema, of pneumonia, etc.

Pathogenesis.—Fatal to white mice, gray mice generally

immune. Guinea-pigs are susceptible in less degree. Probably not very great in human beings.

Morphology.—Occurs as a diplococcus in fours (tetrads). A small organism about 1 μ in diameter.

Biology.—Aerobe, facultative anaerobe. Not motile; grows at room, best at incubator temperature.

Agar Slant.—Slightly elevated, small, round, transparent, grayish colonies.

Gelatin Plates.—Small, round, white, glistening colonies, granular when viewed with the low power. They do not liquefy the gelatin.

Bacillus pyocyaneus.

Habitat.—Pus, tubercular sputum, skin, feces, dust, etc.

Pathogenesis.—Guinea-pigs and rabbits injected subcutaneously with a virulent culture may die within thirty-six hours with inflammatory edema and purulent infiltration of the tissues at the site of the inoculation. Intraperitoneal inoculation may lead to general purulent peritonitis and death.

Bouillon.—Growth appears as a diffuse cloudiness increasing to form a heavy deposit which is at first gray and after a few days forms the characteristic green color.

Agar Slant.—Thick, white, moist, elevated growth within forty-eight hours. The media, even at this time, in the vicinity of the growth may be tinged with green. The medium becomes darkened as the culture ages, and finally is a deep opaque green. The surface growth has meanwhile assumed a greenish color and spread to the edges of the tube. By breaking up the agar of an old culture and shaking with chloroform **pyocyanin** is extracted and may be crystallized by evaporation. The green color is due to this pigment, while another agent, **fluorescein,** may be abstracted by alcohol.

Gelatin Stab.—The medium is rapidly liquefied, a funnel-shaped depression taking place, at the bottom of which is a heavy deposit of organisms. The characteristic color is manifested in the cloudy liquefied mass, and is gradually diffused throughout the medium.

Potato.—Slightly elevated, brown or green, viscid moist growth.

Litmus Milk.—Acid reaction and coagulation.

Dunham's Peptone.—Production of indol.

Diplococcus intracellularis meningitidis (Meningococcus).

Habitat.—The meningeal fluid and pus cells in epidemic cerebrospinal meningitis. It receives its name on account of the frequency with which the organism occurs within the cells.

FIG. 55.—DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS.—(*Councilman.*)

Pathogenesis. — Guinea-pigs and mice are slightly susceptible, dogs and rabbits less so. The disease has been produced in the goat by inoculation within the dura.

In the human being it is believed to gain access to the meninges through the nasal passages.

Morphology.—A biscuit-shaped coccus occurring in pairs or groups of four, the flattened sides being in apposition. At times single forms may be found. It resembles the gonococcus.

Biology.—Grows poorly or not at all upon artificial media, and only at incubator temperature (37° C.). Non-

motile. Cultures must be transplanted every fourth day else they will die.

Staining Reaction.—Stains with all the anilin dyes and is decolorized by Gram's method.

Bouillon.—Very feeble growth. May be slight cloudiness and pale gray flocculent precipitate, viscid and small in amount.

Agar Slant.—Delicate, veil-like growth, thin, gray, translucent, and even may appear as slightly elevated, discrete colonies about 2 to 5 mm. in diameter, opaque, gray and viscid.

Gelatin Stab.—May have feeble surface growth and develop in upper one-fourth of stab. No liquefaction.

Litmus Milk.—Growth takes place without coagulation or other visible change in the medium.

Löffler's Blood-serum.—After incubation from twenty-four to forty-eight hours there occurs a vigorous, creamy, elevated, shiny, viscid growth, generally in the form of colonies, which may remain separate or may become confluent and form a smooth even surface. The medium is not liquefied.

Bacteriologic Diagnosis.—The fluid obtained by lumbar puncture may be used for inoculation of Löffler's blood-serum tubes and should be examined microscopically.

Micrococcus lanceolatus.

Synonyms: Fränkel's pneumococcus, Diplococcus pneumoniæ, Micrococcus pneumoniæ crouposæ.

Habitat.—Found by Sternberg in his own sputum, and occurs in about 20 per cent. of healthy throats. Present in about three-fourths of all cases of lobar pneumonia.

Pathogenesis.—Mice and rabbits are killed within forty-eight hours by the intravenous injection of pneumonic

sputum or pure culture. The inoculation with pneumonic sputum is, in fact, the easiest way of obtaining the pneumococcus for experimental purposes. Frequently sputum from healthy throats will, if injected into the ear vein of a rabbit, produce death from septicemia due to the pneumococcus. A pneumonia may or may not be set up. Other animals are less susceptible.

Morphology.—Not constant. It generally occurs as a diplococcus, oval or lance-shaped. In cultures it may grow as streptococcus, forming long or short chains. When it occurs in the body, it is surrounded by a capsule, but in culture this is seldom present.

FIG. 56.—MICROCOCCUS LANCEOLATUS. —(*Williams.*)

Biology.—Anaerobe, facultative aerobe, growth scanty, soon loses its pathogenic powers in culture and dies. Minimum temperature, 25° C.; maximum temperature, 42° C.; optimum temperature, 37° C. Thermal death-point 52° C. for ten minutes. Non-motile (Sternberg).

Staining Reactions.—Stained by all the anilin dyes and by Gram's method. Capsules may be stained by Welch's method (p. 114). Films should be made from heart's blood of an animal dead of septicemia due to the pneumococcus.

Bouillon.—Grows fairly well and settles to the bottom in sand-like colorless grains. The growth is not viscid

and the medium only faintly clouded. The microscope shows the organisms to be frequently arranged in chains.

Agar Slant.—After forty-eight hours in the incubator there appear small, gray, moist colonies about half a millimeter in diameter. They remain discrete.

Gelatin Stab.—No growth at room-temperature.

Potato.—No visible growth. Film preparations show an abundant growth after forty-eight hours' incubation.

Litmus Milk.—Milk is rendered acid in twenty-four hours, the acidity being increased in forty-eight hours. Coagulation takes place.

Bacteriologic diagnosis is best made by animal inoculation, a small amount of sputum being injected into the ear vein of a rabbit, or a piece of pneumonic lung planted subcutaneously. When the animal dies, film preparations of heart's blood will show the characteristic lance-shaped diplococcus surrounded by a broad capsule. The fact that lobar pneumonia may be caused by other organisms, and that the pneumococcus may be found in a large percentage of healthy throats, renders the discovery of the organism in the sputum of little diagnostic value.

Pneumobacillus (Friedländer).

Habitat.—Air, saliva.

Pathogenesis.—Pathogenic for mice, guinea-pigs, and dogs. Rabbits are immune.

Morphology.—A short bacillus with rounded ends, often nearly round. It may grow in pairs or in chains of four, and may be surrounded by a capsule.

Biology.—Anaerobe and facultative anaerobe. It is non-motile and grows best at 37° C., though it will grow at room-temperature.

Staining Reaction.—It stains readily with the anilin dyes and is decolorized by Gram's method.

Agar Slant.—Develops as large, moist, viscid, grayish colonies.

Gelatin Stab.—The growth is more vigorous upon and near the surface, making the "nail-shape" growth first described by Friedländer, but not limited to this organism. The medium is not liquefied.

Potato.—Vigorous, moist, viscid, grayish growth, rapidly covering the whole surface.

Blood-serum.—Abundant, moist, viscid, dirty white growth.

Litmus Milk.—Coagulation does not take place.

Gas production takes place in media containing glucose or lactose.

Gonococcus (Neisser).

The specific cause of gonorrhea. This organism was discovered by Neisser in 1879, but was not cultivated on artificial media until 1885.

Habitat.—Gonorrheal pus.

Pathogenesis.—Animal inoculations are generally productive of no result. "According to Wertheim, purulent peritonitis not causing death is produced in certain animals by the introduction of serum agar containing colonies of the gonococcus" (Park). In the human being not only does the gonococcus produce a specific urethritis, but it may gain access to the joints, causing arthritis of a severe type, or may enter the blood-stream and set up a fatal endocarditis. The evil effects in gonorrheal ophthalmia and the invasion of the Fallopian tubes by the gonococcus are well known.

Morphology.—Occurs generally as a diplococcus or a tetracoccus, but single forms are observed. The shape of the organism has been likened to a bean or biscuit, the flattened sides facing each other, there being a distinct

line which remains unstained. Degenerated forms may become swollen and round. This is noticed particularly in cultures.

Biology.—Aerobe and facultative anaerobe. Grows only at incubator temperature, 37° C.

The gonococcus will not grow upon ordinary media. Perhaps the best medium so far devised for its cultivation is that of Wertheim. This consists of one part of fluid blood-serum and two parts of agar. The agar is liquefied by heat, cooled to 40° C., and added to the blood-serum heated to the same temperature.

FIG. 57.—GONOCOCCUS IN PUS.—(*Williams.*)

The tubes are inoculated with several loopfuls of gonorrheal pus and allowed to cool in a slanting position. The tubes may first be cooled and inoculated by streaking the surface with pus. Human blood-serum is preferable. It may be obtained from the placenta or in the form of hydrocele or pleuritic effusion, and of course aseptic precautions must be taken throughout the entire procedure. Rabbit's blood-serum answers fairly well. Upon the above medium at incubator temperature colonies of gonococci appear within forty-eight hours as small, discrete, semitransparent, grayish-white plaques or oval colonies, the edges triangular, scalloped, and at times presenting projections. The colonies generally remain separate and reach their maximum size on the fifth day.

They soon die, and if it is desired to preserve the culture, they must frequently be transplanted.

Staining Reaction.—Stains with the ordinary anilin dyes and is decolorized by Gram's method.

Bacteriologic Diagnosis.—For clinical purposes conformity to the following rules is considered sufficient evidence upon which to base a positive diagnosis. If the organism is a gonococcus, it will occur: (1) As a bean-shaped diplococcus; (2) most of the organisms will be *within* the pus cells; (3) they will be decolorized by Gram's method. Cover-glass films should be prepared in the usual way and stained in methylene-blue.

If the diplococci are found within the pus cells, Gram's method is used with a fresh preparation. Several varieties of diplococci occurring in the urethra have been described, but they do not possess all the characteristics of the gonococcus as indicated in the above rules.

In acute cases of urethral or ophthalmic gonorrhea the gonoccocus can easily be demonstrated, as it exists in very considerable numbers. When the disease has become chronic, it is very difficult to find the organisms, and a negative microscopic examination is to be taken only as presumptive evidence of little value. But it is just in this class of cases that a positive diagnosis is often of the utmost value, as in contemplated marriage.

In such cases one cannot be too careful to reach a correct opinion. If the discharge is scanty, and the gonococci cannot be found, the patient should be instructed to drink beer, or a stimulating injection may be used to increase the secretion and augment the number of organisms present, when it may become possible to demonstrate the gonococcus. In the urine of gonorrheal patients small white threads may be observed, the *Tripperfaden* of the Germans, which are mucous in character and which

contain pus cells in varying numbers. By sedimentation of the urine we may frequently obtain these threads in quantity and demonstrate the gonococcus in their pus cells by the usual methods.

One should never pronounce a gonorrheal infection cured until repeated microscopic examination, after irritation, has failed to find the specific germs.

Bacillus proteus (Bacterium termo).

Habitat.—Putrefying substances.

Pathogenesis.—When injected in large quantities, this bacillus will kill rabbits and guinea-pigs. In man it has been associated with pus organisms in numerous inflammatory conditions. Bocker considers that it has some causal relation to cholera infantum.

Morphology.—A rod-shaped organism about 0.6 μ wide and 1.2 μ long. It may at times form long hair-like filaments. Spore formation has not been observed.

Biology.—Aerobe and facultative anaerobe. Grows best at room-temperature and is actively motile. Dependent upon the rapidity with which they liquefy gelatin, three varieties have been described: Proteus vulgaris, Proteus mirabilis, and Proteus Zenkeri. The first variety liquefies gelatin very rapidly, the last not at all.

Staining Reaction.—Stains with all the anilin dyes and is decolorized by Gram's method.

Bouillon.—Diffusely cloudy, flocculent, whitish precipitate. · Odor of putrefaction.

Agar Slant.—Vigorous, whitish, opaque, shiny, moist growth, usually with lateral projections from the edges.

Gelatin.—Vigorous, nodular, white, opaque growth with liquefaction differing in degree according to the variety of the organism.

Potato.—Well-defined, moist, nodular growth, usually odor of putrefaction.

Litmus Milk.—Rendered acid and often bleached. Not coagulated. May be odor of putrefaction.

Bacillus coli communis.

Synonyms: Bacterium coli commune, Colon bacillus.

Habitat.—Found in the intestinal tract of normal human beings and animals.

Pathogenesis.—Virulent cultures injected into the peritoneal cavity of a susceptible animal may produce death in from six to eight days. According to Welch, its degree of pathogenicity is not great. It is frequently found in suppurative processes associated with other organisms, and may cause inflammatory lesions. It seems to more frequently gain entrance to the various organs of the body when they have been previously affected by the invasion of other organisms.

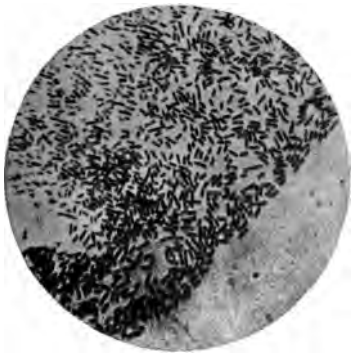

FIG. 58.—BACILLUS COLI COMMUNIS. —(*Williams.*)

Morphology.—The form of the colon bacillus is not at all constant. It may occur as short rods nearly resembling cocci, and at other times form long threads. The classic shape is that of a rod with rounded ends. The diameter is about 5 μ and the length 1 μ to 3 μ.

Biology.—Aerobic and facultative anaerobic and non-liquefying. It does not form spores, though vacuoles may at times be observed. It is flagellated and slightly motile. Growth takes place at room-temperature, but is more rapid in the incubator.

Staining Reaction.—The colon bacillus stains readily with the anilin dyes and is quickly decolorized by Gram's method.

Bouillon.—Diffuse, markedly cloudy, white growth with precipitate. A pellicle may form upon the surface.

Agar Slant.—Vigorous, whitish, elevated, translucent, smooth, shiny growth. The edges of the growth are generally irregular and perhaps puckered.

Glucose Gelatin Stab.—Growth upon the surface and along the whole line of the needle track, with the production of gas-bubbles in the depth of the medium.

Dunham's Medium.—Vigorous growth, usually indol production.

Potato.—Vigorous, dirty white or brownish, elevated, viscid, and granular. (Dependent upon the variation of the kind of potato, we may have considerable variation in the color and vigor of the culture of B. coli communis upon this medium.)

Litmus Milk.—Rendered strongly acid within twenty-four hours and coagulated. (Coagulation usually takes place within forty-eight hours, but may be deferred for a period varying from that time to three weeks.)

Bacteriologic Diagnosis.—See comparison of colon and typhoid bacilli. Bacillus lactis aërogenes and Bacillus pyogenes fœtidis belong to the colon group and are probably varieties of B. coli communis. They are distinguished from the latter by slight cultural differences, and in the case of B. pyogenes fœtidis by the putrid odor produced by this organism in culture.

Bacillus typhosus.

Synonyms: Typhoid bacillus, Bacillus typhi-abdominalis, Bacillus of Eberth.

Habitat.—Intestine.

Pathogenesis.—Lesions similar to those found in the small intestine of man in typhoid fever have been produced in dogs, rabbits, and mice by inoculation with the typhoid bacillus. As a rule, animals are immune to this organism.

There is abundant proof that it is the specific cause of enteric fever, and it has been found associated with inflammatory conditions in the body. The organism can be isolated from the blood of typhoid fever patients in a

FIG. 59.—BACILLUS OF TYPHOID FEVER, STAINED BY LÖFFLER'S METHOD TO SHOW FLAGELLA.—(*Fränkel and Pfeiffer.*)

certain small proportion of cases, and evidently often travels by the blood-stream to portions of the body distant from the intestines, in the Peyer's patches of which the initial lesion occurs.

It has been quite conclusively demonstrated that the typhoid bacillus may under favorable conditions be a pus-producer, and it has been found in pure culture in abscesses of the liver, spleen, and kidneys, in septic meningitis, and in other pyemic conditions.

Morphology.—Variable in character. The typhoid bacillus may exist as a short, oval, coccoid form; more commonly as short rods with rounded ends or more slender thread-like forms. The morphology differs considerably in the same species grown upon different media. The average measurements of the typhoid bacillus are 1 μ to 3 μ in length and 0.5 μ to 0.8 μ in width (Park).

Biology.—Aerobe and facultative anaerobe, non-liquefying, actively motile bacillus. It is provided with numerous long flagella which project in every direction from the body of the organism and when stained give it a mossy appearance. Some of the shorter forms seem to have but a single flagellum. Spore formation by the typhoid bacillus has not been demonstrated, but vacuolation of the protoplasm has been frequently observed. Optimum temperature, 37° C.; maximum temperature, 46° C.; minimum temperature, 10° C.

Staining Reaction.—The typhoid bacillus does not readily take stains, and as a precautionary measure it is well to heat the dye used by holding the slide or cover-glass film on which a quantity of the stain has been dropped over the bunsen flame until steam is given off. Dilute carbol-fuchsin is one of the most useful stains for this organism. The flagella may be stained by special methods. (See page 114.) By Gram's method the typhoid bacillus is rapidly decolorized.

Bouillon.—Vigorous growth producing a diffuse cloudiness with heavy white precipitate and perhaps the formation of a pellicle.

Agar Slant.—Vigorous, pale gray, slightly elevated translucent, shiny, moist growth.

Glucose Agar.—Growth, no gas production.

Gelatin Stab.—Growth more pronounced upon the surface, feeble along the needle track, no liquefaction.

Gelatin Plate.—Small colonies which upon the surface are bluish-white with a serrated border. The deeper colonies are darker in color, smaller, and under the low power are slightly granular.

Potato.—Usually an abundant though invisible growth, which may be demonstrated by microscopic examination of a film preparation. Under some circumstances a visible white layer is produced.

Litmus Milk.—Very slight acid reaction within twenty-four hours. *Coagulation never takes place.*

Dunham's Solution.—Rather feeble growth; no indol produced.

CULTURAL AND OTHER DIFFERENCES WHICH SERVE TO DISTINGUISH THE TYPHOID FROM THE COLON BACILLUS.

TYPHOID BACILLUS.	BACILLUS COLI COMMUNIS.
Flagella eight to eighteen in number.	Flagella few and short.
In all artificial media growth rather slow and feeble.	Growth more rapid and vigorous.
Litmus milk rendered very slightly acid within twenty hours and never coagulated.	Litmus milk rendered decidedly acid within twenty-four hours and coagulated within three weeks.
Indol production in Dunham's medium does not take place.	Usually produces indol in Dunham's medium.
Gas is not produced in glucose agar.	More vigorous growth with production of gas in glucose agar.
On potato, as a rule, invisible but abundant growth.	On potato vigorous grayish or dirty brown growth.
Widal reaction gives positive results, agglutination of the organisms with loss of motility taking place.	Widal reaction negative. The organisms may clump, but they remain motile.

The cultural differences are so slight and their morphology so nearly identical that the blood-serum test generally spoken of as Widal's reaction is now looked upon as the crucial test in deciding the identity of these two organisms.

Widal Reaction.—The value of this test has been frequently questioned. The consensus of opinion by the best authorities is that in 95 per cent. of the typhoid fever cases the Widal reaction may be obtained at some time during the course of the disease, and that, while the colon bacillus may at times be agglutinated, it does not lose its motility.

There are, however, some restrictions placed upon the above assertion. We cannot expect to obtain the Widal reaction even in true enteric fever, except in a small per-

FIG. 60.—POSITIVE WIDAL REACTION.—(*Da Costa.*)

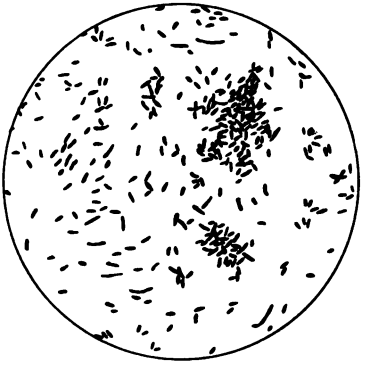

FIG. 61.—PSEUDO-WIDAL REACTION.—(*Da Costa.*)

centage of cases, until the end of the first week, and it is not until the fourth week of the disease that the highest percentage of positive results is reached.

The agglutinizing property may still be retained by the blood of a person who has suffered from typhoid fever months and perhaps years before, though it decreases as time goes on. *The culture used must not be more than twenty-four hours old, and should be actively motile.*

The reaction may be obtained either with dried blood or with blood-serum from a case of typhoid fever.

Dried Blood Method.—Upon a clean glass slip or other piece of glass collect a few drops of blood from a small incision made in the patient's ear or finger-tip, the skin having been previously cleansed with soap and water and wiped dry. The drops should be placed some distance apart and allowed to dry in the air. Blood thus prepared will retain its agglutinizing properties for months. When the test is to be made, five drops of water are added to one of the blood-drops and the two thoroughly mixed with a platinum needle.

With the platinum needle one drop of this mixture is placed in the center of a clean cover-glass, and to this is added with the same loop one drop of a bouillon culture of the typhoid bacillus. (The culture must have been grown in the incubator and must not be more than twenty-four hours old. It is well to examine the culture by the hanging-drop method in order that the motility of the organisms may be fully established.)

The cover-glass is quickly inverted and placed upon a hollow-ground slide the surface of which has, in the neighborhood of the depression, been smeared with a thin coating of vaselin. The cover-glass is gently pressed down upon the vaselin, and an air-tight chamber is thus formed and no evaporation can take place. The slide is transferred to the microscope and examined with the dry high power ($\frac{1}{6}$ or $\frac{1}{8}$ objective), a weak light being used.

The $\frac{1}{12}$ oil immersion is not suitable for this purpose.

If the agglutinizing property of the serum is good, it may be found that the bacteria have already begun to form in groups, and many may have lost their power of motion. In this dilution a positive reaction will ensue within five minutes; that is, there will be marked clumping and loss of motility of the organisms (Park). Within

a short time, say fifteen minutes, all of the bacteria should have become motionless and agglutinated.

Serum Method.—This is perhaps the more scientific method of the two, as the dilution required can be made with more exactness.

A piece of small glass tubing is heated with the bunsen flame and drawn out so that there will be formed two bulbous portions connected by a small tube. The patient's ear having been washed with soap and water, a puncture is made and one of the tubes filled by suction with the blood. The ends of the tubes may now be sealed by holding them for a moment in the flame of a spirit-lamp or with sealing-wax. The blood will soon clot and express several drops of serum. A twenty-four-hour culture grown upon agar is to be used, as from this dilutions can be made more accurately.

Break the tube in which the blood-serum has been collected in the center of the capillary part between the bulbs. The bulb of the empty part is now sucked full of distilled water, twenty-five drops of which are allowed to fall from the recently separated end, in order that they shall be uniform in size with the drop of serum to be used, into a sterilized tube. With the sterilized platinum loop a portion of the culture is removed from the surface of the agar and gently rubbed up with the twenty-five drops of water so that a faint milky emulsion results.

In another test-tube twenty-four drops of distilled water are placed by means of the same tube, and into this from the recently broken end of the capillary tube is allowed to fall one drop of the blood-serum.

With the platinum loop one drop of the emulsion is placed in the center of a clean cover-glass, and, the loop having been sterilized in the flame, a similar drop of the

diluted serum is added. The cover-glass is then inverted over a hollow-ground slide, as in the ordinary hanging-drop preparations, and examined with the dry $\frac{1}{6}$ or $\frac{1}{8}$ objective.

By the above procedure a dilution of 1 : 50 is obtained. Sometimes the reaction as described under the dried blood method will take place in a few moments, but with this dilution of course a longer time is required for the manifestation of the agglutinizing property than would be the case if a lower dilution had been used. Dilutions of 1 : 20 and 1 : 10 may be made in the same way and their results observed. The time required for agglutinization and loss of motility varies with the dilution used.

If a positive result is obtained with a dilution of 1 : 50 within two hours, it is sufficient to warrant the diagnosis of typhoid fever. The higher dilution seems to be advisable, for there is less liability to error, it being established that serum from normal blood may produce the clumping and immotility of the bacteria if used in very low dilution.

One should always make a control preparation from the same emulsion used with the suspected typhoid serum. This is done by using one's own or other normal serum diluted to the same strength of the serum to be tested and combined with a drop of the emulsion and observed under identical conditions.

Bacillus lactis aërogenes.

This organism belongs to the colon group, and is probably a variety of B. coli communis. It is distinguished from the latter by its plumper form, more vigorous gas production, and abundant growth upon ordinary media, and by its being non-motile.

Bacillus of Glanders (B. mallei).

Habitat.—In the lesions and discharges of glanders.

Pathogenesis.—Asses, mules, horses, goats, cats, dogs, swine, guinea-pigs, and field-mice are susceptible to infection by B. mallei. Cattle are entirely immune, as are white mice and domestic mice.

Man is frequently infected, generally through contact with horses suffering from the disease. The infection is generally through some abrasion of the skin, and there follows a local inflammation with a subsequent involvement of the neighboring lymphatics, which soon suppurate. A general infection may now take place, glanders nodules appearing upon the mucous surfaces of the nose accompanied by a universal involvement of the lymphatics of the body. Suppuration of the skin, joints, and muscles may ensue (Levy and Klemperer). When in animals the disease attacks the skin, it is called farcy.

Glanders and farcy are classed among the infective granulomata. The nodules are similar histologically to those produced by the tubercle bacillus, and consist of epithelial cells, leukocytes, and perhaps giant cells lying in a connective-tissue stroma. It may not be possible to demonstrate the bacilli in the sections of the nodules.

Morphology.—A small bacillus with rounded ends 0.2 to 0.4 μ in width and from 2 μ to 3 μ in length. Rarely short threads are formed.

Biology.—Aerobic and facultative anaerobic and non-motile. Maximum temperature, 42° C.; optimum temperature, 37° C.; minimum temperature, 25° C. There is still some question as to whether or not the B. mallei forms spores, but the consensus of opinion is that it does not. The so-called spores are areas of protoplasm similar to those non-staining portions frequently observed in organisms which are known to be non-spore-bearing.

Staining Reaction.—The bacillus stains readily with all the anilin dyes, but gives up the color with equal facility. On this account it is stained in section with difficulty and does not retain the stain with Gram's method. Good preparations may be made by staining films with hot carbol-fuchsin and washing quickly in water.

Bouillon.—Diffuse, cloudy, whitish growth which in time forms a slimy precipitate.

Agar Slant.—Rather feeble, translucent, white, moist, slimy growth.

Gelatin Stab.—Feeble growth along needle track without liquefaction. (Liquefaction may occur in a slight degree after considerable time.)

Potato.—At the end of forty-eight hours in the incubator a vigorous, raised, moist, translucent, slimy, yellowish growth, which has an appearance not unlike honey. By the end of the eighth day the growth will have become opaque and of a reddish-brown color.

Litmus Milk.—Acidified and coagulated.

Blood-serum.—Vigorous, sliny, opaque yellowish growth. No liquefaction of the medium.

Bacteriologic Diagnosis.—The characteristic growth on potato together with its morphologic characteristics render the diagnosis easy. B. pyocyaneus and the spirillum of cholera may produce a similar growth upon potato, but each rapidly liquefies gelatin.

If doubt as to the nature of the organism exists, a male guinea-pig should be inoculated with the pus from a suppurating lymph-node, the excretion from the nostrils, or a culture made from the suspected case. Inoculation should be made with a hypodermic syringe directly into the peritoneal cavity, care being taken to make the puncture in the median line to avoid the seminal vesicles, for

either organism injected into these organs may produce a lesion similar to those produced by the B. mallei.

If the infected material contains the bacillus of glanders, the scrotum of the guinea-pig will begin to enlarge, probably on the second day. The swelling may be deferred some time longer, but should be marked by the end of the fourth day. This reaction is characteristic. The animal will die in the second or third week with all the symptoms of glanders. Great care should be taken in dealing with animals infected with glanders, as many instances are on record of accidental infection of laboratory workers, and there have been some fatal results.

Bacillus of Malignant Edema.

Habitat.—Wide distribution, soil, water, and dead animals and putrefying substances.

Pathogenesis.—Pathologic to animals—the horse, dog, goat, sheep, mice, rabbits, guinea-pigs, etc. The guinea-pig is usually selected for laboratory experiments because of its susceptibility to this organism.

Morphology.—A large rod form with rounded ends, about 1 μ thick and 3 μ to 10 μ in length. It frequently forms long threads both in tissue and in culture.

If a guinea-pig is inoculated with the bacillus of malignant edema or with garden-earth in which the organism exists, death generally takes place in from twenty to forty-eight hours. If the animal is examined immediately after death, there will be found at the site of the inoculation an inflammatory edema, with perhaps a small amount of gas, and microscopic examination will reveal swarms of bacilli and their spores in the fluids of the locality. They will not be found in the blood for the reason that, being anaerobic, it is impossible for them to grow there during life.

If the animal is not autopsied until some hours after death, it will be found that the bacilli have invaded the blood and multiplied there in enormous numbers.

The bacillus of malignant edema is pathogenic to man and produces a local edema at the site of inoculation and a general toxemia which may be fatal. Infection of the human being is fortunately very rare.

Biology.—A strict anaerobe, motile and spore-bearing. The oval spores are frequently situated in the center of the

FIG. 62.—BACILLUS OF MALIGNANT EDEMA SHOWING SPORES.—(*Williams.*)

organism, causing it to bulge at this point. Optimum temperature, 37° C.

Staining Reaction.—Stains by the usual anilin dyes and is decolorized by Gram's method.

Bouillon.—Diffusely cloudy, whitish growth, which later forms a precipitate.

Agar Slant.—Small glistening colonies, whitish and of irregular form. Examination with low power (⅔ obj.)

shows these colonies to consist of a mass of threads which interlace in the most intricate manner and which at the periphery form delicate radiations.

Glucose Gelatin.—Vigorous growth with liquefaction and formation of gas-bubbles. All cultures of the bacillus of malignant edema produce a fetid odor that is intensely disagreeable.

Bacteriologic Diagnosis.—In any suspected case of malignant edema examinations of the body-juices taken from the point of infection should show the organism and spores. These are sufficiently characteristic to lead one to suspect the true nature of the disease at once, though a positive diagnosis cannot be made with the microscope alone.

Cultures should be made in the following manner: Melted glucose agar tubes should be inoculated with the suspected material and subjected to a temperature of 80° C. for half an hour. This will kill all organisms present, but will not kill the spores of Bacillus œdematus malignus. The tubes are now incubated under anaerobic conditions, or if the medium is in sufficient quantity it may be possible to secure a deep growth by ordinary measures. If any further doubt exists, a guinea-pig should be inoculated.

Bacillus of Tetanus (Bacillus tetani).

Habitat.—Garden earth and feces of herbivorous animals.

Pathogenesis.—Horses, goats, sheep, mice, guinea-pigs, and cattle are very susceptible to the bacillus of tetanus, while dogs and fowls seem to be immune. It is pathogenic to man and generally produces a fatal result. The organisms multiply only at the point of inoculation, and even here may exist in small numbers. In experimental tetanus death may occur without apparent multiplication

of the bacilli, from the toxin produced by the organisms at the point of inoculation.

It has been proved that when spores freed from the toxin are introduced into the living body, death does not take place. If some irritant, as lactic acid or a foreign body, or some bacterium, as pyocyaneus, be planted in the wound, the spores of tetanus become pathogenic and produce the usual results. The toxin of the bacillus of

FIG. 63.—BACILLUS OF TETANUS, SHOWING SPORES.—(*Williams.*)

tetanus is the most poisonous substance known to science. It has been estimated that 0.23 milligram of tetanus toxin would produce death in a human being of average weight.

Morphology.—When free from spores, the tetanus bacillus is a slender rod, with rounded ends, about 0.3 μ to 0.5 μ thick, and from 3 μ to 5 μ long. At times it forms threads. When spore formation is present, the organism is of characteristic shape, having an enlarged extremity

in the center of which the spores lie. These are known as drumstick forms.

Biology.—The tetanus bacillus is anaerobic, but will grow in the presence of a small amount of oxygen. It is actively motile in an atmosphere of hydrogen, but motility ceases when the bacilli are brought in contact with oxygen. Maximum temperature, 42° C.; optimum temperature, 37° C.; minimum temperature, 14° C. It forms spores which are at one end of the rod and are extremely resistant to heat and cold and chemical agents.

Staining Reactions.—Bacillus tetani stains evenly and well with the anilin dyes and retains the stain by Gram's method.

Cover-glass films may be stained in the following manner to show the spores:

1. Stain with carbol-fuchsin, heating until the fluid steams, for five minutes, and adding new stain from time to time to compensate for evaporation.
2. Wash in water.
3. Decolorize for one minute in a 2 per cent. solution of sulphuric acid in water.
4. Wash.
5. Stain one minute with a saturated aqueous solution of methylene-blue.
6. Wash in water.
7. Blot with bibulous paper. Dry and mount in balsam.

The spore should be bright red, the body of the bacillus blue.

Bouillon.—Slight turbidity, not characteristic.

Gelatin Stab.—Characteristic growth which consists of linear development along the needle track with pointed radiations into the surrounding medium, the so-called "fir tree" growth. During the second week liquefaction

begins and the bacilli fall to the bottom of the cavity thus formed.

Gelatin Plates.—About the fifth day small whitish colonies, having a dense center, and under the low power appearing to be composed of fine threads in intricate formation, which project from the periphery in delicate lines, giving a feathery appearance to the colony. Slow liquefaction follows.

Glucose Agar Stab.—Growth similar to that in gelatin except that it is not so pronounced and fragmentation of the medium soon takes place owing to the abundant gas-formation. In the cultures there is present a disagreeable odor as of burnt cloth.

Bacteriologic Diagnosis.—The usual method of isolating Bacillus tetani is that of Kitasato. A tube of glucose agar filled two-thirds with the medium is melted, cooled to 42° C., and inoculated with pus or body-juice from the wound supposed to contain the organisms. A considerable quantity of the material should be taken in order to insure securing tetanus bacilli or their spores. The tube is cooled and kept at 37° C. for twenty-four hours, when it is submitted for one hour to a temperature of 80° C., which kills all organisms present but leaves the tetanus spores alive, as they easily resist this degree of heat.

From this material fresh tubes are inoculated and grown under anaerobic conditions.

If after cultivation doubt still exists as to the nature of the organism, a mouse should be inoculated, when, if the culture is Bacillus tetani, the animal will die, showing characteristic symptoms of the disease.

Bacillus aërogenes capsulatus.

Habitat.—Has been found postmortem in a number of

cases in which there has been marked gas development prior to and after death, also in a few cases of emphysematous cellulitis.

Pathogenesis.—Slightly pathogenic to guinea-pigs and pigeons. There is reason to believe that under some circumstances it may be pathogenic to man.

Morphology.—A large bacillus 3 to 6 μ long and frequently inclosed in a capsule. Threads and chains are sometimes formed.

Biology.—Anaerobic, non-motile, and non-spore-bearing. Grows best at 37° C.

Staining Reactions.—Stains with anilin dyes and by Gram's method.

Bouillon.—Diffuse white cloudiness, later forming a sediment.

Gelatin Stab.—Whitish growth, with little or no liquefaction.

Glucose Agar Stab.—Grayish-white growth, with plentiful gas production.

Agar Plate.—Whitish colonies, having peripheral hair-like radiations.

Litmus Milk.—Acid reaction, firm coagulation, and many gas-bubbles.

Potato.—Growth may be invisible or feeble, moist, and white.

Bacteriologic Diagnosis.—Inject the suspected material into the ear vein of a rabbit and after five minutes kill the animal by a blow on the head and place the body in the incubator for twenty-four hours. If Bacillus aërogenes capsulatus is present, abundant gas formation will take place throughout the body, and microscopic examination will show the large encapsulated organisms universally present.

Bacillus pestis bubonicæ. (Bacillus of Bubonic Plague.)

Habitat.—The lesions of bubonic plague.

Pathogenesis.—Pathogenic for man, guinea-pigs, rats mice, monkeys, and other small animals. The rat is particularly susceptible, dying after injection of even small quantities of plague-infected material.

The classic symptoms are enlargement of the inguinal and other lymphatics, which may go on to suppuration, and septicemia, which generally proves fatal. Infection at times takes place through the lungs with pneumonic symptoms.

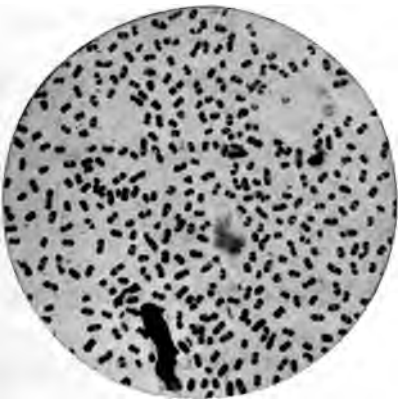

FIG. 64.—BACILLUS OF BUBONIC PLAGUE.—(*Williams.*)

Morphology.—Exceedingly variable. They may appear as short rods, often curved, or as short plump forms suggesting diplococci, and at times as distinct threads.

Biology.—Aerobic and non-motile. Spores are not formed. Grows best at 37° C., but thrives at room-temperature.

Staining Reactions.—Takes the anilin dyes readily and, particularly with methylene-blue, shows deeply stained areas at the ends of the rods (polar staining). It is decolorized by Gram's method. According to Levy and Klemperer, the cultural characteristics are as follows:

"Bouillon is rendered diffusely turbid. If, however, it be inoculated with a coherent bacterial mass from an agar culture, the bacilli develop at the bottom of the tube, while the overlying fluid remains clear. In this way a

growth is obtained that is suggestive of streptococci. On agar plates after twenty-four hours delicate dewdrop-like colonies form that appear, after the lapse of forty-eight hours, as grayish points whose border is slightly iridescent. At times a number of large colonies are seen among the smaller. In agar stab cultures a tough mucoid coating develops, the water of condensation is rendered turbid, but no membrane forms. In Löffler's blood-serum the same developments take place as in agar streak culture. Milk is a poor medium, and is not coagulated. Upon potato a scanty, whitish-gray coating forms at a temperature of 37° C. In culture-media containing sugar the plague bacillus does not generate gas. It does not form indol either in bouillon or in peptone water."

Bacteriologic Diagnosis.—This is rendered easy by microscopic examination of films made from injected glands, the bacilli showing the characteristic polar staining. Cultures and animal inoculations should be resorted to as confirmatory positive evidence.

Bacillus anthracis.

Synonyms: Bacillus of splenic fever, anthrax bacillus.

Habitat.—Lesions of the disease, hides and wool of infected animals.

Pathogenesis.—Sheep and cattle are highly susceptible, as are horses, mice, guinea-pigs, and rabbits. In man there exists a moderate degree of susceptibility and the disease usually appears as a local manifestation, *malignant pustule*, from infection of an abraded surface, or as anthrax of the lungs caused by breathing the dust from infected wool, *wool-sorter's disease.*

It may be rapidly fatal, but the cases usually recover. The organisms are not found in the blood until a short

time before death, but soon after death multiply in the vessels to enormous numbers.

Morphology.—It is the largest of all pathogenic bacilli, 1 μ to 5 μ thick and 5 μ to 10 μ long. The bacilli vary considerably in size under different circumstances, being longer in culture than when grown in the body of an animal. On artificial media they frequently form long

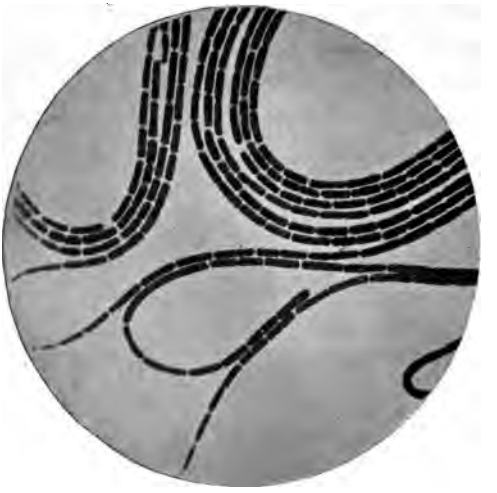

FIG. 65.—BACILLUS OF ANTHRAX, IMPRESSION-PREPARATION.—(*Günther.*)

chains, in which case individual rods have square and at times concave ends.

Biology.—Aerobic and facultative anaerobic, non-motile, and spore-bearing. Maximum temperature, 45° C.; optimum temperature, 35° C.; minimum temperature, 12° C. When grown at 43° C., it loses its virulence after a few generations and does not form spores. The spores form only in the presence of oxygen, but never in the body of an animal. This is probably owing to the presence of

carbonic acid gas, which retards spore formation. The spores are extremely resistant to heat, cold, and chemical reagents.

Staining Reaction.—Stains with the usual dyes and by Gram's method. Spores may be stained by carbol-fuchsin (see p. 153).

Bouillon.—Vigorous growth in shreds and flakes which fall to the bottom and make a dense, ropy sediment.

Agar Slant.—Vigorous, whitish, raised, dry, opaque growth.

Gelatin Plate.—Small white colonies develop in from twenty to thirty-six hours. When viewed with the low power, these are seen to consist of a hair-like mass of twisted threads which project from the edges in wavy lines. The colony looks not unlike a piece of wool, the center dense and opaque, the periphery consisting of threads which frequently form loops. Rapid liquefaction ensues and the colony floats upon the surface as a pellicle.

Potato.—Dense, opaque, abundant, dry, grayish growth.

Litmus Milk.—Coagulated and peptonized.

Bacteriologic Diagnosis.—The size and other characteristics render the determination of anthrax easy. Blood from the ear vein of an animal that is suspected to have died of anthrax may be examined microscopically and by culture. The hay bacillus (B. subtilis) somewhat resembles it, but is not pathogenic. In anthrax in a human being microscopic examination of fluid from the area of inoculation will often reveal the organisms. If doubt exists, animal inoculation should be resorted to.

Influenza Bacillus.

Habitat.—The influenza bacillus has not been observed except in the secretions occurring in the air-passages in influenza.

Pathogenesis.—Among the lower animals monkeys are slightly susceptible to the influenza bacillus and rabbits are so to a less degree.

Morphology.—A very small rod form with rounded ends which sometimes take the stain more readily than the center of the organism. Average size, 0.2 μ thick and 0.5 μ to 1.5 μ in length. In old cultures they may produce longer forms.

Biology.—A strict aerobe, non-motile and non-spore-bearing. Maximum temperature, 42° C.; optimum temperature, 37° C.; minimum temperature, 26 ° C.

Staining Reactions.—The influenza bacillus does not stain readily. Löffler's solution answers fairly well, but better results may be obtained with carbol-fuchsin which has been diluted with 10 parts of water. In this stain smears should be allowed to lie for ten minutes. The preparation is then washed in water, dried, and mounted in balsam.

The influenza bacillus is decolorized by Gram's method.

Cultivation upon ordinary media is very difficult or impossible. The organism grows well on media streaked or mixed with blood. Agar slant tubes are generally used, their surfaces having been smeared with the blood of an animal such as a rabbit. The best way to accomplish this is to kill the animal by a blow on the head and reflect the skin of the thorax. The chest is now opened with a pair of sterile scissors, the heart grasped with a pair of sterile forceps and cut off at its base. The detached and bleeding heart may now be used to smear the tubes and plates, which should be put in a cool place until wanted.

To obtain pure cultures from a case of influenza, Pfeiffer recommends that the bronchial sputum or a portion of pneumonic lung in a case of influenza pneumonia be tri-

turated in a mortar with 2 c.c. of bouillon until a homogeneous emulsion results. Blood agar tubes and plates are inoculated by distributing a portion of this material over their surfaces with the platinum loop.

Control smears should be made upon the ordinary agar in the same manner. The emulsion is made for the purpose of diluting the organisms so that discrete colonies may form, and also to dilute the hemoglobin present so that no influenza bacilli will grow upon the plain agar tubes.

The inoculated tubes are incubated for twenty-four hours, when the colonies of the influenza bacilli will appear as dewy drops, usually so small that they can be seen only with the low power of the microscope. These colonies may reach the size of a pin-head provided they are widely separated.

Bacteriologic Diagnosis.—Depends upon the microscopic and cultural characteristics as outlined. Frequently in the discharge from the nasal and bronchial mucous membranes the influenza bacilli may be obtained in nearly pure culture.

Bacillus of Diphtheria.

Synonyms: Klebs-Löffler bacillus, Bacillus diphtheriæ.

Habitat.—In the membranes and mucous tissue upon which they occur in cases of diphtheria and in a small percentage of healthy throats.

Pathogenesis.—While the diphtheria bacillus is pathogenic in varying degrees to most animals, they are singularly free from the disease except when inoculated with the organisms for laboratory purposes. Guinea-pigs, rabbits, and cats are among the more susceptible animals; horses are less so, and are used for the production of antitoxin.

The organism does not multiply to any great extent in the tissues of animals, and usually is found only at the site of the lesions upon the mucous membranes and in the secretions of the mouth and pharynx in cases of diphtheria.

They have in a few cases been procured in pure culture postmortem from the spleen, kidneys, and lungs; but probably when they reach the internal organs it is through the medium of the blood to which they gain access shortly

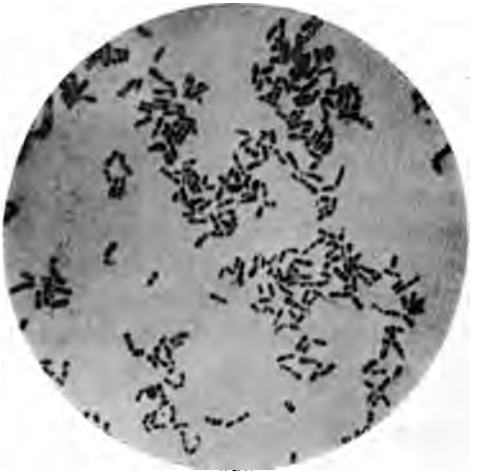

FIG. 66.—BACILLUS OF DIPHTHERIA.—(*Fränkel and Pfeiffer.*)

before death. The various lesions of the deeper structures and the paralysis of diphtheria are undoubtedly due to the toxins produced by the organisms at the site of growth upon the mucous membrane of the air-passages.

Subcutaneous injections of virulent cultures prove rapidly fatal to guinea-pigs. The organisms will be found at the site of the inoculation, which is edematous and hemorrhagic, and may at times be recovered from one or more of the various organs.

Morphology.—Extremely variable in form, the diphtheria bacillus may appear as a short, thick rod with rounded ends, or several organisms may be side by side, their ends being pointed so that they resemble cigars; again, they assume a club form, one end being tapering, the other swollen and round, and at times both ends are enlarged—the so-called dumb-bell form. They vary from 0.5 μ to 1 μ in thickness and from 1 μ to 6 μ in length.

Biology.—The diphtheria bacillus is aerobic and facultative anaerobic. Maximum temperature, 40° C.; optimum temperature, 37° C.; minimum temperature, 20° C. It does not form spores nor does it liquefy gelatin. When kept in the dark, it may in the dried state live for months.

Staining Reactions.—The Klebs-Löffler bacillus is stained by Gram's method. Among the anilin dyes, methylene-blue in the form of Löffler's solution is chiefly used for staining this organism.

LÖFFLER'S METHYLENE-BLUE SOLUTION.

Saturated alcoholic solution of methylene-blue,	30 c.c.
Solution of potash in water 1:1000,	100 c.c.

This acts quickly and is a very useful stain for many other bacteria. Cover-glass preparations are fixed by passing through the flame and submitted to the action of the stain for five minutes, dried, and mounted in balsam. Ordinary aqueous solutions of methylene-blue also act well.

The diphtheria bacillus stains in a peculiarly irregular manner with methylene-blue, and this is one of its distinguishing features.

It will be found that under the $\frac{1}{12}$ oil immersion many of the bacilli are crossed by clear bars, indicating unstained areas, which give them a striped appearance.

Others will be solidly stained, while some will be more deeply stained at the ends, the middle being lighter in color. Smears from the throat usually show a more even staining of the bacilli, though the spotted appearance is still marked.

Preparations from blood-serum cultures are more characteristic than those made from the ordinary media. In old cultures on any medium the organisms stain poorly.

Neisser has published a stain which he claims will differentiate the true from the pseudo-diphtheria bacillus, the polar bodies in the former being distinctly stained by it.

NEISSER'S STAIN FOR POLAR BODIES.

Alcohol (95 per cent.),	20 parts.
Methylene-blue,	1 part.
Distilled water,	950 parts
Acetic acid (glacial)	50 parts.

Dissolve the methylene-blue in the alcohol, add the water, and then the acetic acid.

No. 2.

Bismarck-brown,	1 part.
Distilled water (boiling),	500 parts.

Dissolve and filter.

The technique of the stain is as follows:

The organisms must be grown at incubator temperature upon Löffler's blood-serum, and must not be under twelve nor over eighteen hours old; a fifteen-hour culture is preferred.

Cover-glass films are made and fixed in the usual manner; they are stained for five seconds with solution No. 1, washed in water and stained for five seconds with solution No. 2, washed in water, dried, and mounted in balsam. According to Neisser, if the culture is one of diphtheria bacilli, many of the organisms will contain round, deep-blue bodies, the remainder of the rod being stained brown;

while if the culture is one of pseudo-diphtheria, only a few of the polar bodies will be found.

According to Park, this method is not reliable, many virulent cultures failing to show the bodies, while at times they may be found in quantity in the non-virulent variety.

Bouillon.—At twenty-four hours within the incubator there is a slight turbidity with a small amount of whitish precipitate. Subsequent growth is not characteristic.

Glucose Bouillon.—Growth is similar to that in plain bouillon. The medium becomes acid within the first or second day, and after a few days becomes alkaline again.

Agar Slant.—Usually a scanty growth, the colonies may be discrete or coalesce and form a thin, white, transparent layer.

Glycerin-agar Plates.—Within twenty-four or forty-eight hours small, translucent, grayish-white colonies appear. Under the low power these are irregular, often laminated and granular.

Gelatin Stab.—Feeble growth, no liquefaction.

Blood-serum (Löffler's).—Within twenty-four hours small raised plaques appear, opaque and grayish in color. If not too close together, they continue to grow, and by the third or fourth day may have formed a continuous layer. The colonies generally retain their integrity, at least in part.

In the cultures made from the throat the colonies of diphtheria bacilli after the first twelve hours are larger than those of streptococci and not so large as those of staphylococci, if any be present.

In the cultures several days old the pearl-gray tint of the colonies may be replaced by a yellowish hue.

Potato.—An invisible growth results, as may be proved by microscopic examination of cover-glass preparations.

Litmus Milk.—It is a favorable medium, but is not changed by the growth.

Bacteriologic Diagnosis.—Pseudo-diphtheria Bacilli: In a small percentage of cases of pseudo-membrane of the respiratory passages bacilli are found which conform both in their morphology and cultural characteristics to diphtheria bacilli, but which are not pathogenic to guinea-pigs and which in culture produce no toxin. These are believed to be diphtheria bacilli which have for some reason lost their virulence.

There are other bacilli occurring in the normal respiratory passages, the conjunctivæ, and even upon the skin which conform in morphology to the diphtheria bacillus, but which differ slightly from it in culture and which fail to produce toxins in culture and are generally not pathogenic to animals. Diphtheria antitoxin does not protect animals from the action of these organisms, which have been called xerosis bacilli. The following table gives the differences generally considered to exist between the true and false diphtheria bacilli. It may be difficult at times to designate to which class a given culture belongs. Certainly the two are closely related, and the xerosis bacilli may have sprung originally from the diphtheria bacilli.

TABLE SHOWING CULTURAL AND OTHER DIFFERENCES BETWEEN TRUE AND FALSE DIPHTHERIA BACILLI.

BACILLUS DIPHTHERIÆ.	BACILLUS PSEUDO-DIPHTHERIÆ.
Glucose bouillon: Acid within the first forty-eight hours, later alkaline.	*Glucose bouillon:* No acid reaction.
Agar: Feeble growth, small colonies, limited extension from line of inoculation.	*Agar:* Vigorous growth, large grayish-white colonies which soon become elevated, thick, and extensive.

Potato: Invisible growth.

Löffler's blood-serum: Small, pearly colonies becoming larger after twenty-four hours.

Neisser's stain: Cover-glass preparations from fifteen hours' growth on Löffler's serum show numerous polar bodies.

Animal inoculation. Pathogenic to guinea-pigs in amounts depending upon the virulence of the culture used.

Potato: Vigorous, visible growth.

Löffler's blood-serum: More vigorous growth, colonies soon become larger and more elevated and softer than those of the diphtheria bacillus.

Neisser's stain: Cover-glass preparations from fifteen hours' growth on Löffler's serum show very few or no polar bodies.

Animal inoculation: Not pathogenic to guinea-pigs.

Cultures should always be made in cases of suspected diphtheria. The various boards of health in our large cities have adopted for this purpose a small box of wood in which are two test-tubes, one containing Löffler's blood-serum, the other a sterile swab. The method for preparing the blood-serum has already been given (see p. 87). The swab is made by flattening one end of a stout iron wire about six inches long by beating it with a hammer, and around this end a small piece of absorbent cotton is tightly wound.

The swab is placed in an ordinary test-tube the mouth of which is closed with a cotton plug, and the whole is sterilized for one hour at a temperature of 150° C. The swab is used to inoculate the culture-tube, it having been found better adapted for the purpose than the platinum loop.

Inoculations are made as follows: The swab is removed from its tube and the cotton plug is placed in the neck of the tube.

The swab is held by its free end and its cotton portion is allowed to touch nothing but that part of the throat from which cultures are desired.

The patient, having been placed in a good light, is directed to open his mouth and the tongue is depressed with a suitable instrument so that a clear view of the throat and pharynx may be secured.

The cotton swab is rubbed several times over the patches of pseudo-membrane that are present upon the pharynx and fauces.

The cotton plug is now removed from the blood-serum tube and held between the fingers, as is usual in making inoculations, and that portion of the swab which has been in contact with the throat is rubbed several times across the blood-serum. This should be done with gentle pressure so that the serum will not be broken up.

The plug having been replaced in the serum tube, the swab is replaced in its tube, and both are put in the box, which now may be sent to the laboratory or transported to one's office for investigation.

The inoculatoin thus made should be incubated for at least six hours, when it may be examined with the low power of the microscope and cover-glass smears may be made and stained by Löffler's solution in the manner already described.

According to Park, who, in connection with the New York Board of Health, has had large experience in this special work, smears made from cultures usually show "either an enormous number of characteristic Löffler bacilli with a moderate amount of cocci, or a pure culture of cocci either in pairs or short chains (streptococci). In a few cases there will be an approximately even mixture of Löffler bacilli and cocci, and in others a great excess of cocci.

"Besides these, there will be occasionally met preparations in which with the cocci there are mingled bacilli more or less resembling the Löffler bacilli. These bacilli,

which are usually of the pseudo-diphtheria type, are especially frequent in cultures from the nose."

Inoculations may be made in like manner from the pseudo-membrane in the nose, vagina, and conjunctiva.

Smears upon glass slips may be made with the cotton swab *after* the tube has been inoculated, otherwise the tube would be contaminated; and such preparations, having been fixed by heat (see p. 108) and stained with methylene-blue, may show bacilli upon which an immediate diagnosis may be made. One should be cautious, however, in making a diagnosis from smears obtained directly from the throat, as otherwise he is liable to fall into error.

There are cases in which the characteristic bacilli may be obtained almost in pure culture by this method, and under such circumstances a positive diagnosis is warranted. It is wise to make only a provisional diagnosis from the smear or to suspend judgment until the culture has developed.

Mixed Infection.—As has already been indicated, a double infection in diphtheria is by no means infrequent. The streptococcus is the more common organism accompanying the Klebs-Löffler bacillus, but it may be associated with staphylococci, pneumococci, and the colon bacillus.

A pseudo-membrane may be formed in the throat or nasal passages through the agency of the streptococcus alone.

Persistence of Diphtheria Bacilli.—It has been proved that diphtheria bacilli in virulent form may remain for several weeks in the throats of persons who have recovered from diphtheria, so that they may be a source of danger to those with whom they come in contact. They also have been found in apparently healthy throats in persons who have been in attendance upon cases of diphtheria and who have not developed the disease.

Recapitulation of Methods of Examination in Cases of Suspected Diphtheria.

Cultures:

1. Inoculation of slant tubes of Löffler's blood-serum by means of a sterile cotton swab.
2. Incubate tube from six to twelve hours, preferably the latter.
3. Examine for minute pearly colonies of diphtheria bacilli, which may just be visible after twelve hours in the incubator.
4. With a sterile platinum loop remove a small portion of the growth to a drop of water on a clean cover-glass held in the Cornet forceps.
5. Spread the drop evenly with the shank of the platinum wire.
6. Dry in the air.
7. Pass three times through bunsen flame.
8. Stain ten minutes with Löffler's solution of methylene-blue.
9. Wash in water.
10. Blot with bibulous paper.
11. Dry by passing rapidly through bunsen flame.
12. Mount in balsam.
13. Examination with $\frac{1}{12}$ oil immersion lens.

Examination may show:

1. Löffler's bacilli almost in pure culture.
2. Streptococci almost in pure culture.
3. About equal mixture of streptococci and Löffler bacilli.
4. Staphylococci, pneumococci, colon bacilli, or other organisms are infrequently found alone or mixed.
5. A guinea-pig may be inoculated to determine the virulence of the culture.

Smears from the throat:

1. Made after inoculation of culture-tube, using the same cotton swab, on cover-glass or glass slip.
2. Dry in air.
3. Fix by passing three times through bunsen, with cover-glass, until uncomfortably warm to finger, with slip.
4. Stain ten minutes with Löffler's methylene-blue.
5. Wash in water.
6. Blot with bibulous paper.
7. Dry by passing rapidly through bunsen flame.
8. Mount in balsam.
9. Examination with $\frac{1}{12}$ oil immersion lens may show:
   (*a*) Large numbers of characteristic diphtheria bacilli.
   (*b*) Streptococci in nearly pure culture.
   (*c*) A mixture of organisms from which no conclusion can be reached.

Bacillus tuberculosis.

Habitat.—Lesions of tuberculosis and pus from same; dust from houses, public conveyances, halls, etc.

Pathogenesis.—It has been computed by competent observers that tubercle bacilli cause one death in every seven in the human family. They are pathogenic in a high degree to many of the domestic animals, as cattle, pigs, and rabbits. Guinea-pigs are highly susceptible to tuberculosis, and for this reason are of great value in studying the disease in the laboratory.

Intraperitoneal injection of bouillon culture, even in minute quantity, will produce a rapid and progressive tuberculosis in the guinea-pig, the animal dying in from four to eight weeks. Autopsy will show characteristic tubercles in the peritoneum, lymph-glands, liver, and perhaps the kidneys. There may be cheesy pneumonia,

and cheesy degeneration of the lymph nodes and of areas in the various organs is common.

The tuberculosis of fowls will be treated under the proper head. Monkeys and other animals in captivity frequently suffer from tuberculosis. Field-mice and cats may be infected by doses of virulent cultures. Dogs are practically free from spontaneous infection, but the disease may be produced in them by inoculation with large doses of bouillon culture of the tubercle bacillus.

Morphology.—Slender delicate rods, often slightly curved, usually 2.5 μ to 3.5 μ long and about 0.3 μ thick. Organisms reaching 6 μ in length are sometimes observed in both culture and in the sputum from tubercular patients. In the sputum the bacilli may be single or several may lie side by side or may be arranged in an irregular lump.

FIG. 67.— BACILLUS TUBERCULOSIS FROM A PURE CULTURE.—(*Fränkel and Pfeiffer.*)

Biology.—The tubercle bacillus is aerobic and non-motile. It has been thought that it forms spores, but this has been disproved. Maximum temperature, 41° C.; minimum temperature, 29° C.; optimum temperature, 37° C. Their powers of resistance are greater than those of most organisms. They resist drying for several weeks and cold does not affect them. In the dry state they may not be killed by a temperature of 100° C. sustained for several hours, but when

moist this degree of heat kills them in a few minutes. Direct sunlight is rapidly fatal to their vitality.

Staining Reaction.—The tubercle bacillus takes the anilin dyes poorly unless they are combined with a mordant. When once stained, it is difficult to decolorize them. This fact is of the utmost importance, as it enables us to stain the tubercle bacillus by methods which serve to differentiate it from all other bacteria. This organism stains irregularly, as does the diphtheria bacillus, there being portions of the protoplasm that remain uncolored, so that the stained bacilli frequently have a beaded appearance. These unstained areas have been thought to be spores, but it has been proved that this is not the case.

Culture Characteristics.—The tubercle bacillus is unfortunately very difficult to grow on artificial media. Upon the ordinary media, with the exception of blood-serum, it will not grow except in the presence of glycerin. At best, growth is exceedingly slow, first appearing at the fourteenth day in the thermostat. When the tubercle bacillus has once been grown on artificial media, subcultures are more vigorous and develop quicker.

Blood-serum.—This is the best medium upon which to grow the organism. To obtain a culture direct from tubercular sputum is very difficult, owing to the fact that other organisms present outgrow the tubercle bacillus. By inoculating the guinea-pig with the sputum, and after the lapse of six weeks planting bits of tubercular tissue from the peritoneum of the animal on blood-serum, pure cultures may be obtained. After fifteen days in the incubator the colonies begin to appear as small, elevated, whitish, dry areas which increase slightly in size during the next few days, and may, if close together, coalesce and form longer colonies.

Subcultures from the above will be more luxuriant,

and after several generations will form the characteristic elevated, dirty-white, irregular, hard, dry, wrinkled patches that we are wont to see in cultures of this organism.

Glycerin-agar.—When the tubercle bacillus has been cultivated upon blood-serum until the fifth generation, it may be transplanted upon glycerin-agar, where it will grow with comparative rapidity and with considerable vigor, often covering the surface of the slant with a thick, wrinkled, dry, grayish layer, perhaps a millimeter in thickness.

Glycerin Bouillon.—If scales of tubercle bacilli be transplanted to a flask of this medium in such a manner that they float upon the surface, rapid growth ensues, so that the thick, wrinkled pellicle may reach the sides of the flask and even grow upward for a considerable distance upon its surface. The bouillon below meanwhile remains perfectly clear.

Potatoes.—According to Levy and Klemperer: "Upon potatoes whose lower extremities project into a solution of glycerin 5 per cent. and sodium chlorid 5 per cent. the tubercle bacilli grow exceedingly well. Upon the surface of the potato they form a dense deposit. The glycerin solution remains clear and is covered with the well-known coating." Trudeau recommends the use of potato cylinders which have been soaked for some hours in glycerin bouillon.

Bacteriologic Diagnosis.—The early diagnosis of any tubercular process is of the most vital importance. The lesions may occur in any part of the animal economy, and it is therefore frequently necessary to examine the various secretions and excretions of the body. As cattle often become tuberculous, it is desirable to examine milk, flesh, and butter intended for consumption for the purpose of detecting the tubercle bacilli.

Sputum.—In the human being the lungs are usually the site for the primary focus in tuberculous affection, in which case the tubercle bacilli will be found in the sputa.

There are several methods of staining the tubercle bacilli in sputum. Of these, the Ziehl-Neelsen method alone will be given, as it is the more frequently used and gives a beautiful picture with certainty even in the hands of the novice.

Three solutions are necessary:

No. 1.

Fuchsin, ... 1 gm.
Alcohol (95 per cent.), ...10 c.c.
Dissolve and add a 5 per cent. aqueous solution of carbolic acid 90 c.c.

This solution will keep well and is a beautiful general stain. This is known as Ziehl's solution of carbol-fuchsin.

Caution: Acid fuchsin must not be used for this stain.

No. 2.

Sulphuric acid, ... 5 c.c.
Water, ...95 c.c.

No. 3.

Methylene-blue, ... 1 gm.
Water, ...100 c.c.

The sputum coughed up in the morning should be collected in a clean wide-mouthed bottle. When it has been brought to the laboratory, it is well to empty the contents of the bottle into a clean Petri dish which is placed over a piece of black paper.

With the platinum loop purulent portions and any small cheesy white masses that may be seen are transferred to a clean *slip* (do not use a cover-glass) and the whole spread in a thick smear over the central third of its surface. This smear should be rapidly dried over the bunsen flame, taking care not to heat the slip to the point

where it will crack or to such a degree that it will burn the sputum. It is well to heat the glass in a pair of strong thumb-forceps until it is uncomfortably warm to the fingers when brought in contact with them.

As soon as the sputum is thoroughly dried, *and while the slip is still hot*, cover the smear with a liberal amount of carbol-fuchsin (solution No. 1) and allow it to remain for two minutes. The glass should be hot enough to cause the solution to smoke slightly, and if this is not observed, it is well to heat the slip with the stain upon it until the steam begins to rise. Wash thoroughly under the tap—

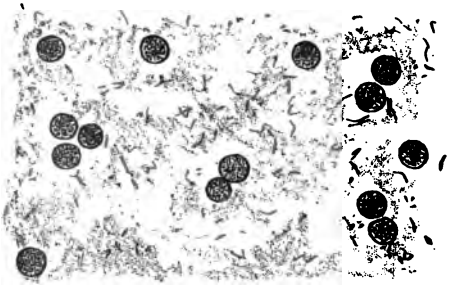

FIG. 68.— BACILLUS TUBERCULOSIS IN SPUTUM, STAINED WITH FUCHSIN AND METHYLENE-BLUE.

a few seconds will suffice for this step—and immerse in 5 per cent. sulphuric acid (solution No. 2) in a Petri dish for two minutes, moving the slip about or rocking the dish occasionally.

Wash for a moment under the tap to remove the acid. The smear should now be pale pink. If it is still red, replace it in the acid solution to still further decolorize it. After a half minute remove and wash as before. Stain for half a minute with solution No. 3, wash under the tap, wipe the under surface, ends, and edges of the slip with a towel, blot the smear with bibulous paper, and dry thor-

oughly by passing the slip three times rapidly through the bunsen flame. A drop of cedar oil is now put directly upon the stained smear and it is examined with a $\frac{1}{12}$ oil immersion objective. A cover-glass is not necessary, and slips thus used may be cleansed by boiling in soap and water and may be used over many times. If after examination it is decided to keep the preparation, a drop of balsam and a cover-glass are added. The cedar oil may previously be blotted off, but this is not necessary.

Table of Steps in Staining Sputum for Tubercle Bacilli.

1. Make thick smear on glass slip.
2. Dry rapidly over bunsen flame.
3. Stain two minutes with carbol-fuchsin (solution No. 1). The stain should smoke. If necessary, heat until this occurs.
4. Wash under the tap.
5. Immerse in a 5 per cent. aqueous solution of sulphuric acid (solution No. 2).
6. Wash under the tap.
7. Stain half a minute with methylene-blue (solution No. 3).
8. Wash under the tap.
9. Wipe bottom, ends, and edges of slip with towel.
10. Blot smear with bibulous paper.
11. Pass a few times through the bunsen flame to dry.
12. Put on a drop of cedar oil and examine (without a cover-glass) with a $\frac{1}{12}$ immersion lens.

Of course, the smear may be made upon a cover-glass, but in the author's opinion the slip is far preferable for routine work.

Caution: Too much care cannot be taken in handling tubercular sputum. It should never be allowed to stand

where flies have access to it, and if any is accidentally spilled it should be wiped up with a cloth saturated with a strong bichlorid solution which should always be kept about the laboratory to be used in disinfecting. Bottles which have contained sputum should be disinfected by boiling, or if they are valueless, burned in the furnace.

The result of the Ziehl-Neelsen method is that, if the tubercular bacilli are present, they will be stained red, while the other bacteria, having given up the red stain in the acid solution, and the nuclei of pus corpuscles and other cells are stained blue.

There are several bacteria which take the carbol-fuchsin stain and which do not give it up when submitted to the action of an acid. These are the bacillus of leprosy, of syphilis, and the smegma bacillus. It is usually quite easy to exclude each of these organisms, none of which will be found in the sputum. The smegma bacillus exists in the unctuous secretions of the genitals and may gain access to the urine and be mistaken for the tubercle bacillus which it somewhat resembles morphologically. It can readily be differentiated from the latter organism by following the procedure below outlined.

Examination of the urine may be made in suspected tuberculosis of any portion of the genito-urinary tract. A quantity of *morning* urine having been obtained, it is sedimented with the centrifugal machine or by allowing it to stand for twelve hours in a conical glass. A considerable amount of sediment is removed with a pipette and transferred to a clean glass slip, upon the center of which it is spread in a rather thick layer. The smear is now *slowly* evaporated by heating the slide held in the forceps in the bunsen flame. When the smear is thoroughly dry, staining is proceeded with as in sputum, except that the preparation is submitted to the action of 95 per cent.

alcohol for five minutes, *after* the smear has been decolorized in the sulphuric acid solution and *washed*. The alcohol decolorizes any smegma bacilli that may be present, but will not affect the stain of the tubercle bacilli. Tubercle bacilli in the urine are usually difficult to demonstrate because of the fact that they exist in small numbers. They are usually found in clumps and at times in quantities. If the first examination of the urine or sputum is negative as to tubercle bacilli, the second, and indeed several more examinations, should be made if necessary before basing an opinion upon the absence of the organism. It is well to bear in mind that while we may make a *positive* diagnosis of tuberculosis by means of the microscope, we are *not* justified in making a negative diagnosis if the tubercle bacilli are not found. In the latter case the conclusion reached must be the Scotch verdict, "not proven."

Examination of pus from abscesses and necrotic bone supposedly due to the action of the tubercle bacillus usually yields no result. Whether the organisms are present in exceedingly small numbers, or, what is more probable, are by their environment in some way changed so that they do not stain by the usual methods, we do not know, but the fact remains.

In a case in the above category it is almost useless to endeavor to find the tubercle bacillus with the microscope, but animal inoculation gives us a ready and efficient means of diagnosis. A guinea-pig is inoculated, preferably intraperitoneally, with some of the suspected material. If at the end of six weeks the animal has not died, it should be killed and examined for the characteristic tubercular lesions, the presence of which proves the process from which the original pus was taken to have been tubercular.

Feces may be examined by spreading the material on a piece of glass and holding this over a black surface. With the platinum loop the small, lentil-shaped, whitish flakes are picked out, spread upon a slip or cover-glass, and subsequently treated in the manner prescribed for sputum.

Milk.—Milk should be sedimented by allowing it to stand in a conical glass or by the centrifuge and the sediment spread upon a slip and dried slowly by heat, which will at the same time fix it. It must then be immersed for five minutes in a Petri dish of chloroform to remove the fats present, else it will not stain. It is removed from the dish and the adherent chloroform allowed to evaporate. Subsequent procedures are as with sputum.

Avian Tuberculosis.—Fowls are frequently affected with tuberculosis, but it has long been known that there exist certain cultural and other differences between the bacilli found in the tubercular lesions of fowls and those from mammalian tuberculosis.

The experiments of Nocard seem to have demonstrated that the bacillus of mammalian tuberculosis and that of avian tuberculosis are varieties of the same organism, and each may under proper circumstances assume the properties and the characteristics of the other.

To Stain the Tubercle Bacillus in Sections.

1. Stain for half an hour in carbol-fuchsin.
2. Wash in water.
3. Decolorize in 5 per cent. sulphuric acid solution for five minutes.
4. Wash in water.
5. Wash in 95 per cent. alcohol until sections become pale pink.
6. Wash in water.

7. Böhmer's hematoxylin one-half minute.
8. Wash in water five minutes.
9. Alcohol 95 per cent. to dehydrate five minutes.
10. Carbol-xylol one minute.
11. Mount in balsam.

Bacillus of Leprosy.

Habitat.—The lesions of leprosy.

Pathogenesis.—While a few observers have claimed to have produced experimental leprosy in animals, the consensus of opinion is that the lepra bacillus is pathogenic only for man, in whom it produces two forms of lesions—the *nodular* or *tubercular leprosy* and the *anesthetic leprosy*. The former is the more common affection, and is manifested by nodular enlargements occurring in the skin of the face, backs of the hands and feet, mucous membranes of the mouth and larynx, and other situations.

The development of the nodule is slow and is due to a low-grade irritation set up locally by the bacilli, which seem incapable of producing a toxin of sufficient power to affect the more distant portions of the organism. Structurally, the nodules consist of connective tissue, leukocytes, and epithelial cells, some of which may be multinucleated without resembling the giant cells so common in tuberculosis. Caseation of the nodule does not occur.

In the *anesthetic* form the lesions are found in the nerves, and consist of a chronic inflammatory process with consequent degeneration of nerve-fibers and atrophic disturbances in the skin and membranes of the body.

Morphology.—The lepra bacillus closely resembles the tubercle bacillus in size and form. It may possess the same beaded appearance due to unstained areas, and some forms may have pointed, others swollen club-shaped ends.

Biology.—Numerous and repeated attempts to cultivate

the lepra bacillus have failed. Cultures have in some cases been obtained from cases of leprosy, but the organisms failed to produce the disease in animals and morphologically differed from the lepra bacillus found in the tissues.

Staining Reaction.—Bacillus lepræ stains more readily than does the tubercle bacillus, taking up the anilin colors quite readily. When stained with carbol-fuchsin, the bacilli retain the color more tenaciously than most organisms, but give it up more readily than the tubercle bacillus —a fact of considerable diagnostic importance.

They are stained by Gram's method.

Bacteriologic Diagnosis.—Pus or scrapings from nodules in tubercular leprosy may be spread upon cover-glasses or slips and stained in the same manner as in searching for tubercle bacilli in sputum, except that decolorization in 5 per cent. sulphuric acid solution should only last for one-half minute. Sections of the nodules of leprosy may be stained by the same method by allowing them to remain in carbol-fuchsin overnight, washing in water until no more stain is given out, and decolorizing for thirty seconds in 5 per cent. sulphuric acid. The time required to decolorize the sections will depend largely upon their thickness, and is a matter of personal judgment.

The tissues are now washed, stained with Böhmer's hematoxylin and mounted, after the usual manner, in balsam. The bacilli are red and the nuclei of the cells are purple.

In section the bacilli are found in enormous numbers within and around the cells of the granulation tissue.

Bacillus lepræ is so closely allied morphologically to the tubercle bacillus that the two cannot with certainty be differentiated by microscopic examination alone.

It must not be forgotten that leprosy is in this country a rare affection. The characteristic numbers and group-

ing of the bacilli in the tissues, together with the clinical aspect of the case, should render diagnosis easy.

Bacillus of Syphilis.

In 1884 Lustgarten described a bacillus, which he found in the primary sore and internal lesions of syphilis, as being the causative agent of the disease. The organism is said to be a slender rod somewhat resembling the tubercle bacillus.

The bacillus has not been cultivated upon artificial media, neither are animal inoculations with syphilitic tissue containing it productive of results.

Upon the whole, the evidence at present brought to bear upon the subject is far from conclusive, so that as a diagnostic factor we must consider the organism valueless.

Actinomyces bovis (Ray Fungus).

Habitat.—The pus and lesions of actinomycosis and probably upon wheat and rye.

Pathogenesis.—In cattle the disease is usually manifested by tumor-like masses occurring in the lower and more rarely in the upper jaw (lumpy jaw), or by enlargement and induration of the tongue, known as "woody tongue." The disease tends to remain local and spreads by continuity of tissue. Necrosis of the bone, softening, and abscess formation are the usual results, the abscess pointing and breaking externally rather than in the mouth.

In man the symptoms are quite different, there being little tendency to tumor formation. The disease is usually characterized by an insidious and wide-spread chronic inflammatory process, which is finally productive of suppuration. The fungus spreads by continuity of tissue and also through the lymph and blood channels and may in-

vade any portion of the body. In the internal organs the lesions are particularly apt to suppurate, and small cavities may break into others of similar size, so that the tissue becomes honeycombed with abscess.

The pus has a greenish hue, and in it may readily be found the masses of fungus, which appear as yellowish,

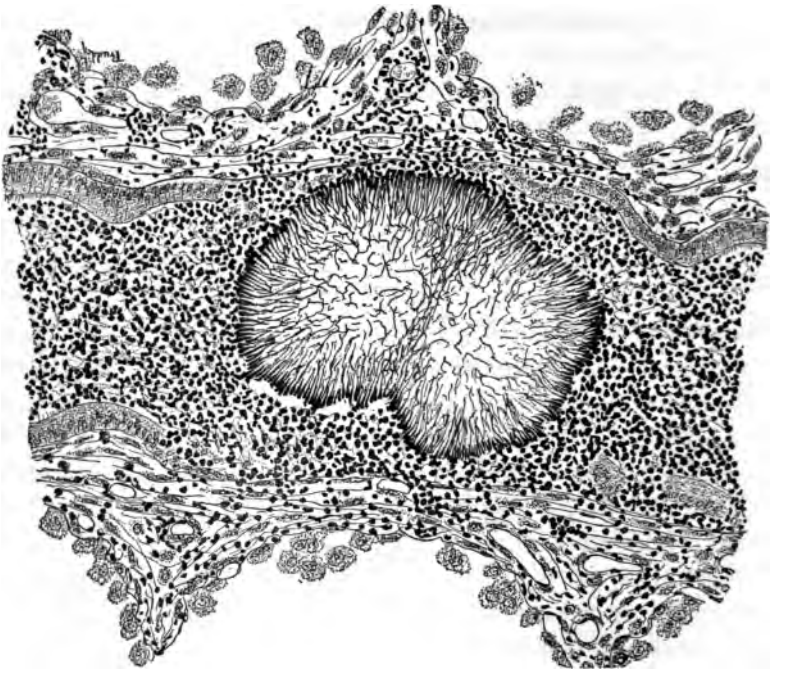

FIG. 69.—ACTINOMYCES BOVIS.—(*Delafield and Prudden.*)

translucent granules, many being as large as the head of a pin.

The fungus may be obtained in pure culture, and, according to Wolff and Israel, it is possible to produce the disease experimentally in rabbits, guinea-pigs, and sheep by inoculation with agar or egg cultures. Other ob-

servers have obtained negative results. The disease has been produced by the inoculation of an animal with the pus of actinomycosis.

Morphology.—The ray fungus is classified by Kruse as belonging to the streptothrices, a class intermediate, as it were, between the bacteria and the molds. The microscopic characteristics are comparatively complex. If the granular bodies which exist in the pus of actinomycosis, and which measure from 0.5 mm. to 2 mm. in diameter, be examined under the low power, they are seen to consist of a mass of threads which radiate from a common center, and each of which may terminate in a club-like extremity. The filaments are 5 μ in diameter, and may be of considerable length and often branch.

Coccus-like bodies may be formed from the threads by segmentation of the protoplasm, the sheath inclosing it often remaining unchanged and visible. Other spherical forms seem to arise in a manner similar to that which takes place in the formation of gonidia in the molds, and to be, in fact, the spores of the organism. In cultures the clubs may be absent.

Staining Reactions.—The threads and spherical bodies stain readily by Gram's method and by solutions of the ordinary anilin dyes. The club forms are best stained by using Gram's method, and, as a contrast stain, picric acid or rubin, as recommended by Muir and Ritchie.

Biology.—There is still some doubt as to whether there may not be more than one species of actinomycosis. The organism with which Wolff and Israel experimented was anaerobic, and was obtained from human subjects, while that usually described is aerobic, and it is not possible with this growth in culture to produce the disease in animals.

The anaerobe grows best upon agar or egg media at a temperature of 37° C., producing a visible growth about

the fifth day. The development is slow and there are found upon the surface of the medium small droplets which are at first translucent but later become opaque. These usually remain discrete, but may coalesce and form a coating over the surface of the media.

Bacteriologic Diagnosis.—Pus from a suspected case should be collected upon glass slips and spread in a thin layer. If the actinomycosis is present, the granules may be observed by the unaided eye by holding the slip to the light, or the low power (⅔ obj.) may be used with advantage.

The smears may be stained by Gram's method, which will demonstrate the threads and spherical bodies. To bring out the club-like extremities, one must use a counterstain, as already stated. In the tissue the organism may also be demonstrated by the above method. It is almost impossible to confound the ray fungus with anything else.

Spirillum choleræ asiaticæ (Comma Bacillus).

Habitat.—Stools of persons suffering from Asiatic cholera.

Pathogenesis.—The lower animals are under ordinary conditions not subject to cholera, and therefore experimental inoculation cannot be expected to produce positive results. Extensive experiments have been performed by numerous observers, and after rendering the alimentary tract alkaline and restraining peristalsis by large doses of opium it has been found possible to produce in guinea-pigs a condition similar to cholera by injecting into the stomach virulent cultures of comma bacilli. Ninety per cent. of the animals die and exhibit postmortem congestion of the small intestine with swarms of cholera bacilli in the copious fluid contents.

The disease has been experimentally produced in man both designedly and by accidental inoculation in handling cultures in the laboratory. From all the cases pure cultures of the spirillum of cholera have been recovered. The organism therefore conforms to Koch's law, and it is universally admitted by bacteriologists to be the causative agent in the production of Asiatic cholera.

Morphology.—A comma-shaped bacillus with rounded

FIG. 70.—SPIRILLUM OF CHOLERA, FROM A CULTURE ON STARCHED LINEN.—(*Fränkel and Pfeiffer.*)

ends. When two comma forms lie end to end, they form an S-shaped vibrio. The organism is a small one, measuring about 0.8 μ to 2 μ in length and about 0.5 μ in width. Many of the forms, especially the younger ones, are straight or only slightly curved. In cultures where conditions are unfavorable there may be found long and wavy threads. These also may be found in the peritoneal cavity of an animal experimentally injected with cholera cultures.

As observed by Koch in cover-glass preparations made from the dejecta of cholera patients, the comma bacilli are frequently arranged side by side with the long axis pointing in one direction, for which reason they have been likened to a school of fish. This is an important diagnostic symptom.

Biology.—A flagellated, actively motile, aerobic and facultative anaerobic spirillum. It rapidly liquefies gelatin and does not form spores. Maximum temperature, 42° C.; optimum temperature, 30° to 40° C.; minimum temperature, 80° C.

Staining Reactions.—The comma bacillus does not retain the stain by Gram's method. It stains with some difficulty with ordinary dyes, but readily with carbol-fuchsin and anilin-water-gentian-violet, particularly when applied with heat.

The flagella may be demonstrated by Löffler's method for staining these processes.

Cultural Characteristics.—Bouillon: Within twelve hours at incubator temperature the medium is universally turbid, and upon the surface is a thin white pellicle composed of comma bacilli which if examined in the hanging-drop will be found actively motile. It is essential that the bouillon be alkaline.

Agar Slant.—Within forty-eight hours there is a heavy, grayish, translucent, moist, glistening coat.

Gelatin Stab.—At the end of twenty-four hours there is a rather feeble growth, which is seen as a white line along the whole extent of the needle track, but growing thinner in the deeper portions. Between twenty-four and forty-eight hours liquefaction begins at the surface, and generally forms a funnel-shaped depression which has the appearance of an air-bubble in the medium owing to the evaporation of the liquid formed. The whole needle

track becomes gradually enlarged by the liquefying process, so that many of the organisms sink to the bottom and form a whitish mass which may be spiral in shape. Under favorable circumstances the surface liquefaction may by the sixth day have reached the sides of the tube. If the gelatin is too stiff, liquefaction will not be so rapid.

Gelatin Plates.—Within forty-eight hours the characteristic colonies begin to appear as small white dots, which, when viewed with the low power, present a rough glistening appearance which has been likened to that of broken bits of glass.

Liquefaction soon begins, and the colony forms about it a cup-like depression into which it sinks. The grayish opaque colony is surrounded by a light rim of liquid medium. After a few days the colonies have become more or less disintegrated and lie at the bottom of the enlarged area of liquefaction.

Potato.—Upon potato medium which has been rendered alkaline by soaking in a 0.5 per cent. solution of carbonate of soda the comma bacillus grows rapidly, usually forming a grayish or brownish layer.

Litmus Milk.—May or may not be coagulated and is rendered slightly acid.

Blood-serum.—At a temperature of 37° C. blood-serum is rapidly liquefied, a vigorous grayish, moist growth taking place.

Peptone.—In Dunham's medium the comma bacillus grows rapidly. Cultures in this medium are used with advantage in testing for indol.

Bacteriologic Diagnosis.—This presents some difficulties from the fact that there are a number of organisms which closely simulate the comma bacillus both morphologically and in culture. Some of these forms are undoubtedly

deviations from a type of the vibrio of Asiatic cholera, while others are distinct species.

The following method of procedure for the diagnosis of the cholera vibrio is taken entire from Park's "Bacteriology."

Plan of Procedure for Biologic Diagnosis of the Cholera Vibrio.

Dejecta (fluid) or intestinal contents of a cholera patient or cholera suspect.

1. Use one drop (one platinum loop) for gelatin plate cultures, making two dilutions. Do this in duplicate or triplicate. Cultivate at 22° C.

2. Inoculate a couple of bouillon tubes and a couple of tubes of Dunham's 1 per cent. peptone solution with one drop of each dilution, and place them in the incubator (37° to 38° C.) for six to eight hours.

3. Examine a drop of the dejecta in the hanging-drop.

4. Examine a drop of the dejecta in stained cover-glass preparation.

5. Make gelatin plates from one drop taken from the surface of the bouillon and peptone solution tubes and cultivate at 22° C.

6. As soon as the plates (see 1 and 5) are sufficiently developed (thirty-six to forty-eight hours), fish the suspected cholera colonies and use the material for the following procedures:

7. Inoculate six or eight peptone tubes (1 per cent. peptone, 0.5 per cent. sodium chlorid in distilled water) and place them at once in the incubator. Note the time.

8. Examine the hanging-drop for form, size, and motility (and arrangement).

9. Make stained cover-glass preparations and examine.

10. Then try indol reaction with the same tubes.

11. While these tubes are incubating use material from the suspected colonies on the plates (1 and 5) for hanging-drop cultures.

12. Meanwhile make stained cover-glass preparations from other colonies of suspected cholera on the plates (1 and 5).

13. Make gelatin tube cultures from colonies on plates (1 and 5).

14. Make gelatin tube cultures daily for five or six days, to study shape of growth along the line of puncture to preserve the culture.

Spirillum of Relapsing Fever.

Synonym: Spirillum Obermeieri.

Habitat.—The blood of persons suffering from relapsing fever.

Pathogenesis.—The spirillum of relapsing fever has been successfully inoculated in man and apes, reproducing the disease. It is found postmortem in the spleen and other organs.

Morphology.—A long thin spiral thread having many turns, the length varying from 16 μ to 40 μ and the ends tapering to a point. The long thread is the only form which has been observed, and it evidently does not break into shorter forms, as do other vibrios.

Biology.—Cultivation upon artificial media has not been accomplished, and therefore little is known about the organism. Its constant presence in the blood in cases of relapsing fever, and the fact that inoculation with the infected blood produces the disease in other individuals, substantiate Obermeir's theory as to its being the causative agent of the disease.

Staining Reaction.—It does not stain by Gram's method but takes the ordinary anilin dyes with avidity.

Bacteriologic Diagnosis.—The size and morphology of the spirillum of relapsing fever and its situation in the blood render the diagnosis easy. In fact, there is nothing with which it may be confounded.

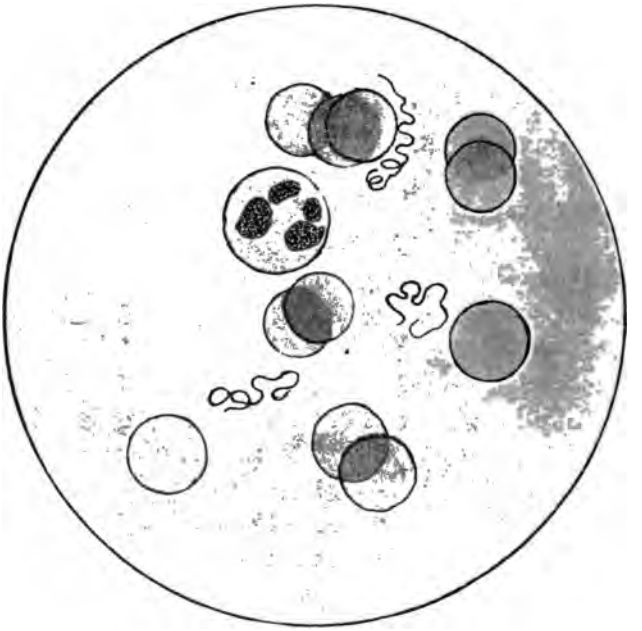

FIG. 71.—SPIRILLUM OF RELAPSING FEVER IN THE BLOOD.—(*Williams.*)

Bacillus icteroides, Bacillus of Yellow Fever.

Habitat.—In the bodies of persons dead of yellow fever.

Pathogenesis.—Pathogenic to rabbits, guinea-pigs, rats, cats, dogs, monkeys, and mice.

In animals dead of infection with B. icteroides there is found postmortem jaundice of the skin, fatty degeneration of the liver, enlargement of the spleen, congestion of the kidneys, and hemorrhagic areas of the gastro-intestinal

mucosa. In man, according to the report of the Commission of Medical Officers to investigate the cause of yellow fever (U. S. M. H. S.), "the infection takes place by way of the respiratory tract, the primary colonization in this tract giving rise to the earlier manifestations of the disease"; this primary infection being followed by a "secondary colonization of the organism in the blood of the patient," thus constituting a septicemia.

Biology.—A motile bacillus with rounded ends, about 2 μ to 4 μ in length and about 0.5 μ thick. It is aerobic and facultative anaerobic and does not liquefy gelatin.

Spores.—Have not been demonstrated.

Bacillus icteroides belongs to the colon group. Bacillus icteroides resists drying for several days, but it is readily killed by the usual germicidal agents or by exposure to direct sunlight.

Staining Reactions.—It is decolorized by Gram's method, but stains by the ordinary anilin dyes.

Cultural Characteristics.—Growth takes place rapidly and there is found neither a deposit nor a pellicle.

Agar Slant.—Upon this medium the growth is quite characteristic. According to Muir and Ritchie: "After twenty-four hours at 37° C. there is a gray, iridescent, and somewhat transparent growth. On being transferred to a temperature of 20° C. to 28° C., this becomes in twelve hours surrounded by a halo of white, opaque, pearly growth of higher level than the central point. In a few days the growth at the lower temperature becomes liquid in character and runs slowly down the medium as a drop of melted paraffin would do."

Gelatin Plates.—Small, round, transparent, granular colonies which do not liquefy the gelatin.

Potato.—A thin transparent pellicle which does not become discolored by age.

Milk.—Coagulation takes place, but only after some weeks.

Bacillus icteroides ferments media containing glucose and saccharose with the production of gas, but has no effect upon lactose which has not been changed by being subjected to too high a degree of heat.

Bacteriologic Diagnosis.—This is a matter of some difficulty, as the organism resembles several of the colon group. It may be differentiated by noting its cultural characteristics and by inoculation of animals, in which it produces icterus, fatty degeneration of the liver and acute parenchymatous nephritis, and acute gastro-enteritis with hemorrhagic areas.

Recent researches have cast considerable doubt upon the validity of the claim that B. icteroides is the causative agent of yellow fever, and the matter must for the time be held *sub judice.*

HYDROPHOBIA, RABIES.

There remains little doubt that hydrophobia is due to the action of a specific organism, but as yet all efforts have failed to isolate such a bacterium. Distinct and constant pathologic lesions have recently been demonstrated in the ganglia of the posterior nerve-roots.

YEASTS.

Yeasts multiply by budding of the parent plant. The offshoots appear as oval, non-nucleated processes, which eventually become detached and grow to adult size. Pathologically yeasts are of but little moment, for though several varieties have been isolated from human beings, the conditions in which they are found are very uncommon.

MYCOSES.

The moulds consist of masses of thread-like cells having a tendency to apical growth. The interwoven and branching vegetative part is called the *mycelium*. From this arise stems, often jointed, which bear the fruit or spores. The moulds possess no chlorophyl.

Many of the moulds are pathogenic, the lesions produced by them being principally limited to the skin.

Favus.—Schonlein in 1839 described as the specific cause of favus a filamentous fungus which was named in his honor *Achorion Schonleinii*. The organism may be isolated and grows freely upon all culture-media, being at first whitish and later becoming yellow. Gelatin is liquefied.

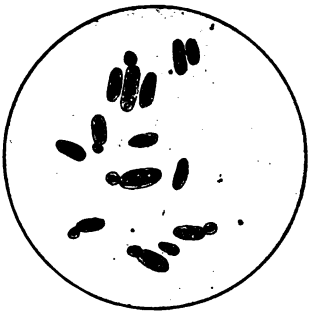

FIG. 72.—YEAST CELLS.—(*Williams.*)

The disease occurs in dogs, cats, and mice, and doubtless these animals prove a source of infection to human beings, though it is not highly contagious.

The microscopic diagnosis is easy. Portions of the crusts formed in the course of the disease should be moistened with water and mounted in glycerin, firm pressure being made upon the cover-glass in order to flatten the mass. There will be seen epithelial cells of a horny nature, and concentrically arranged, unjointed, branching, mycelial threads, which send toward the center of the *favus body* jointed fruit stalks containing spores.

The hairy portions of the body are most frequently

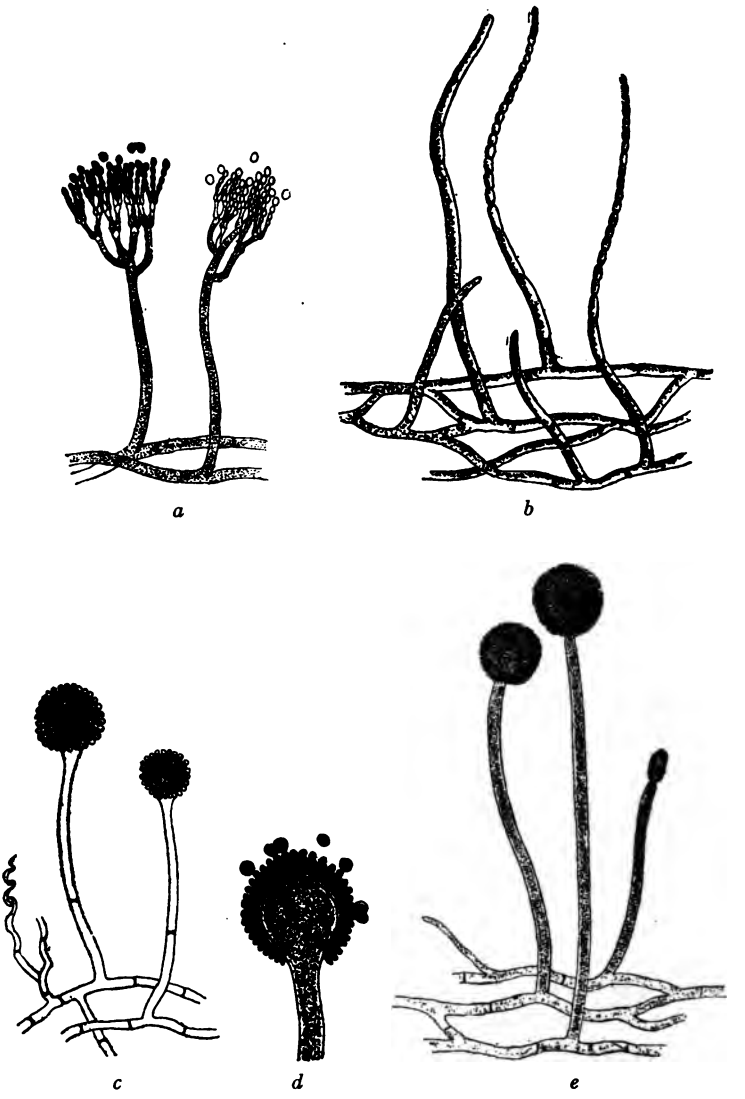

FIG. 73.—MOLDS.

a, Penicillium glaucum. *b*, Oidium lactis. *c*, Aspergillus glaucus. *d*, The same more highly magnified. *e*, Mucor mucedo.—(*Baumgarten.*)

attacked, and in such cases masses of the fungus may be seen invading the bulb and root sheaths of the easily detached hairs.

Herpes tonsurans and a number of parasitic affections of the non-hairy skin are caused by *Trichophyton tonsurans*. "The fungus appears especially in the form of extended, slightly branched, and straight mycelia, whose width is less and whose segments are comparatively much shorter than those of the favus fungus. The conidia of the trichophyton resemble those of achorion, although they are somewhat smaller, and, above all, less numerous." (Levy and Klemperer.)

The trichophyton may be cultivated upon artificial media and differs from the favus fungus chiefly in its more luxuriant growth and more rapid liquefaction of gelatin.

Microscopic examination is facilitated by submitting the scales or hairs removed from the affected area to the action of ether for a few moments to remove the fat and then to a solution of potassium hydroxid. In the latter they should remain for five minutes, and may then be mounted in a drop of glycerin and a cover-glass pressed firmly upon them so that the mass will be rendered as thin and transparent as possible.

Pityriasis Versicolor.—This condition is brought about by the presence in the skin of *Microsporon furfur*, a fungus similar to the trichophyton and achorion. "The fungus masses, which are at once recognizable in the horny lamella removed by means of the finger-nails or a sharp curette and mixed with a 6 per cent. solution of potassium hydroxid upon a glass slide, consist of unusually large and uniform conidia (spores) which form regularly distributed masses, each of thirty or more, and of slightly ramified, short mycelia, which connect the conidia masses with one another." (Levy and Klemperer.)

Thrush.—The thrush fungus, *Oidium albicans*, produces in human beings a local disease attacking mucous membranes, chiefly those lined with pavement epithelium.

It is found more frequently in the mouth or pharynx of nursing children or those adults suffering from wasting diseases (typhoid or tuberculosis). It may invade the larynx, and even the deeper portions of the respiratory tract, and has been found in the stomach and vagina; it is usually limited to the mucosa, but may at times penetrate deeper, involving the submucosa.

Upon acid media the thrush fungus thrives and exhibits the characteristics of a budding fungus. It does not liquefy gelatin.

Microscopic Diagnosis.—A portion of the growth is removed to a clean glass slip, mixed with a drop of glycerin, and a cover-glass superimposed. The growth will be seen to consist of conidia and mycelial threads, the latter of double contour and varying in their dimensions. Frequently the threads are branched and interlacing. The spores are spherical in outline and occur at the ends of the mycelia.

Moulds are not infrequently found in the external auditory canal, particularly following suppurative processes in this locality.

ACTINOMYCOSIS.

This has already been considered. The exact botanical position of Actinomyces bovis is uncertain.

TUMORS.

A tumor is an abnormal new-growth of tissue, not inflammatory in origin, atypical in structure, performing no physiologic function, and possessing no fixed limit of growth.

A tumor, or neoplasm, has its prototype in normal structures, and, according to the cellular theory, arises always from cells similar to its structure. The causation of neoplastic growth is beyond the scope of this book.

The classification below given is adopted from Green's "Pathology."

CLASSIFICATION OF TUMORS.

Mesoblast, ...

I. TYPE OF HIGHER TISSUES.

Type of muscle, ... Myoma.
" " nerve, ... Neuroma.
" " blood-vessels, ... Angioma.
" " lymphatic vessels, ... Lymphangioma.

II. TYPE OF FULLY DEVELOPED CONNECTIVE TISSUE.

Type of fibrous tissue, ... Fibroma.
" " mucus, ... Myxoma.
" " adipose, ... Lipoma.
" " cartilage, ... Chondroma.
" " bone, ... Osteoma.
" " neuroglia, ... Glioma.

III. TYPE OF EMBRYONIC CONNECTIVE TISSUE.

The varieties of sarcoma.

IV. TYPE OF EPITHELIAL TUMORS.

Epiblast and Hypoblast. .....

Papillæ of skin and mucous membranes, ... Papilloma.
Glands, ... Adenoma. / Carcinoma.

V. TERATOMA OR CONGENITAL MIXED TUMORS.

The Myomata.

A myoma is a neoplasm composed in part or entirely of muscular tissue. There are two varieties, one containing unstriped muscle; the other, striated muscle.

Leiomyoma.

Leiomyomata consist of smooth muscle-fibers mingled with a greater or less amount of connective tissue. They are firm nodular growths, frequently encapsulated, and occur in the skin and subcutaneous tissues, where they

arise from the muscular structures in the walls of the blood-vessels, or from the erectores pilorum; they are not infrequent in the intestinal and genito-urinary tracts. The most common form is the so-called fibroma of the uterus.

These tumors may grow to an enormous size, so that they weigh 70 or 80 pounds. They are composed of

FIG. 74.—LEIOMYOMA. × 360.

fibrous tissue and smooth muscle, mingled in various amounts. If the muscle predominates, the tumor is soft and fleshy; while if the fibrous tissue is in excess, the mass may be hard and tough. The latter neoplasms should properly be called leio-myo-fibromata.

A section of leiomyoma shows the characteristic spindle cell of smooth muscle, bundles of which are bound to-

gether by connective tissue and run without law in a variety of directions. The spindle character of the cells can be distinguished only when they are cut in their long diameter; if cut transversely, they appear as round or polygonal cells, some of which contain nuclei.

It will be readily seen that, dependent upon the portion of the cell cut and the direction of the section, a spindle cell may present a great variety of appearances. The student should bear this constantly in mind and endeavor to interpret the picture presented by a section of such tissue. The typical form of a nucleus of a smooth muscle-fiber is that of a rod with rounded ends.

One must at times be very guarded in his diagnosis of tumors from the uterus, as it may happen that a leiomyofibroma may closely simulate a sarcoma; even an expert may be at fault, and it may be that a positive diagnosis cannot be made. This applies chiefly to those instances where small portions of tissue are removed for diagnostic purposes prior to operation.

It must always be borne in mind that sarcoma is rare as a primary growth in the uterus.

The leiomyomata, especially that variety occurring in the uterus, frequently undergo degeneration.

Large calcareous nodules may form in the center of the neoplasm or mucoid or cystic changes may occur.

The leiomyomata are dangerous only on account of size and situation, when by pressure upon vital structures they may produce serious or even fatal consequences.

Rhabdomyoma.

Rhabdomyomata are neoplasms that contain a greater or less amount of striated muscle. It is very seldom that they are found pure, the muscle elements being usually associated with other tissues. These growths are congeni-

tal, and are found in the kidney, testicle, and uterus, and rarely in other portions of the body.

Many of the rhabdomyomata contain sarcomatous elements. These tumors, rhabdo-myo-sarcomata, may occur in the kidneys, and may reach a large size and ultimately prove fatal. Such a growth would present the general characteristics of a spindle-celled sarcoma, while here and there would be found bundles and single muscle-fibers of an atypical character. The muscle-fibers are usually present in small numbers, though they may be found in considerable quantities in one portion of the growth while in another part they may be very few.

These fibers may be quite long and wavy, with smooth edges and well-marked striations, approaching in character an adult, normal muscle-fiber, or may be spindle-shaped, nucleated, and distinctly embryonic in type. Other fibers may have varicosities and hyaline areas and pursue a very irregular course. A sarcolemma has not been demonstrated on the muscle-fibers of the rhabdomyomata.

While the tumors are congenital, they may give no trouble until late in life, and then suddenly take on a rapid and malignant growth.

The rhabdomyomata are among the rarest of tumors.

Neuroma.

A neuroma is a neoplasm composed largely of nervous elements. Two varieties are distinguished.

1. *Myelenic neuomata*, consisting of new-formed medullated nerve-fibers.

2. *Amylenic neuromata* consisting in great part of non-medullated nerve-fibers.

The neuromata are benign, but may be multiple, occurring in large numbers over the whole or part of the

body. They arise from the pre-existing nerves, either peripherally or more rarely at their origin. Their growth may be quite rapid.

Hemangioma.

A hemangioma is a neoplasm consisting chiefly of blood-vessels, some of which are a new-formation, others being

FIG. 75.—CAVERNOUS ANGIOMA OF LIVER. × 100

only dilated abnormal vessels normal to the part. Such a growth is known as a *simple angioma.*

They occur in the skin as *vascular nævi,* and frequently in the liver. They are formed chiefly by the dilatation of existing capillaries with thickening of their walls and subsequent atrophy of the intervening epithelial structures through pressure and loss of nutrition. There are also a few vessels of undoubted recent origin.

Should the vascular cavities break into one another, thus forming large cavities, the growth would be called a *cavernous angioma.* According to Zeigler, cavernous angiomata occur rarely in the kidneys, spleen, uterus, intestines, bladder, and bones.

In section an angioma presents the appearance of atypical blood-vessels filled with blood.

Lymphangioma.

The lymph-vessels may form tumors similar to the hemangiomata. They consist of dilated lymph channels with thickened walls supported by connective tissue, and probably represent changes in structures normally present, though formation of new vessels doubtless occurs to a certain extent.

The tongue is often the site of these tumors (*macroglossia*), and they may less frequently be observed upon the lips, neck, scrotum, and other portions of the skin.

Fibroma.

A fibroma is a neoplasm composed of connective tissue. They may be hard or soft or may occupy a station anywhere between these two extremes. They consist of spindle or branching cells which lie in bundles, the latter having no definite form of arrangement. In the soft varieties the processes of the cells may be quite long and the nuclei comparatively large. The cells of the hard fibromata are spindle-shaped, the nuclei are small with pointed ends. The more recent the growth, the larger the nuclei will be, while in old formations the nuclei of many of the cells may have disappeared, leaving only a wavy, fibrous mass in certain areas of the microscopic field.

Fibromata may occur in any portion of the body where connective tissue is normally found. They spring more

frequently from the denser connective tissues, fasciæ, periosteum, the sheaths of nerves, and subcutaneous tissues. Small fibromata are of not infrequent occurrence in the kidneys. In the mammary gland they form tumors that are often mistaken for malignant growths.

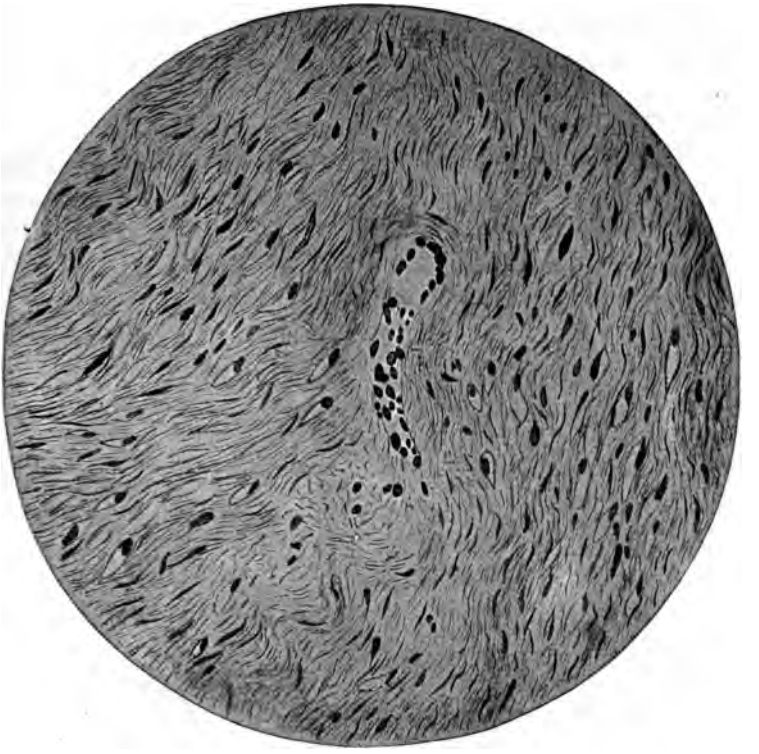

FIG. 76.—SOFT FIBROMA.—(*Coplin.*)

These neoplasms form around the ducts of the gland and constitute the *pericanalicular fibroma* of the breast.

If there are numerous ingrowths of the connective tissue, forming peninsulars which project into the lumen of the ducts, the growth is called *intracanalicular fibroma.*

The fibromata are often encapsulated, and they are entirely benign except when they interfere with the physiology of the organism by pressure or in some purely mechanical way. Usually the blood-supply of the fibromata is scanty. They may undergo calcareous or fatty degeneration.

The so-called fibroma of the uterus has been already referred to under the head of leiomyomata.

Myxoma.

The myxomata are composed of mucous tissue, having

FIG. 77.—MYXOMA. × 100.

their prototype in the umbilical cord and vitreous humor of the eye.

They consist of irregularly shaped cells with rather large nuclei and long, branching, protoplasmic processes which anastomose one with another to form a loose net-

work, the interstices of which are filled with gelatinous semifluid contents. This form of tissue is usually associated with some other variety of tumor, usually of the connective-tissue type.

We may have resulting, *lipomyxomata*, *fibromyxomata*, *chondromyxomata*, and *myxosarcomata*. Myxomatous neoplasms are more frequently found in structures rich in connective tissue, as the periosteum, subcutaneous tissue, skin, the sheaths of tendons, and fasciæ.

Neoplasms are frequently found in the parotid gland consisting of mucous tissue combined with sarcomatous elements—*myxosarcomata*. If, as is often the case, cartilage is also present, the tumor is known as a *myxochrondrosarcoma*. Such growths as contain sarcomatous tissue are always more or less malignant, and on this account dangerous.

Lipoma.

A lipoma is a tumor composed of fat, supported by a connective-tissue stroma. These neoplasms are usually inclosed in a capsule and are frequently lobulated and quite firm.

They may be so soft as to impart a sense of fluctuation, as of a fluid. They are found upon the buttocks, back, neck, and abdomen more frequently than in other situations, but may occur in any of the softer structures of the body.

Microscopically, they consist of a connective-tissue reticulum containing fat. They may occur in connection with mucous tissue (*lipomyxomata*), or the fibrous tissue may be excessive, in which case the tumor would be called *fibrolipoma*. They are subject to degenerative changes, calcification, necrosis, and sloughing. They may reach an enormous size, but are essentially benign.

Chondroma.

A chondroma is a tumor composed of cartilage, generally of the hyaline variety. Usually these neoplasms are developed in situations where cartilage normally exists—about the ends of the bones, in the lungs and trachea; but they may occur in the parotid gland, testis, or muscles.

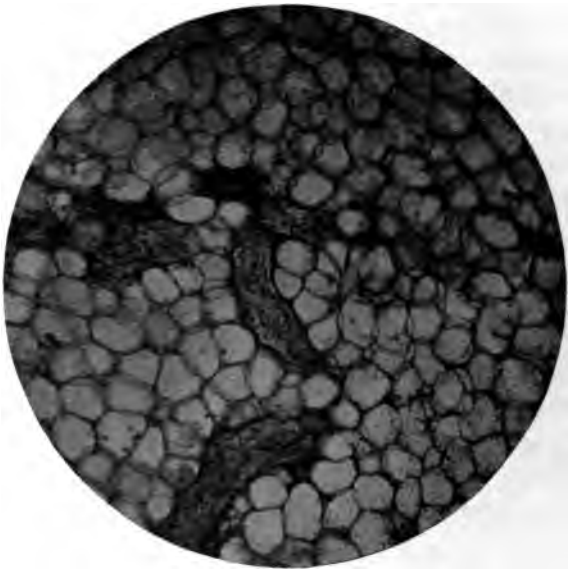

FIG. 78.—LIPOMA. × 100.

They present as rounded, smooth, or irregularly lobulated bodies, firm or elastic to the touch.

Not infrequently they are associated with mucous tissue (*chondromyxomata*), bone (*osteochondromata*), or sarcomatous elements (*chondrosarcoma*).

They may undergo mucoid or cystic degeneration, or become calcified or bony. Microscopically they present, to greater or less extent, the appearance of normal carti-

lage; or if associated with other tissues, islands of cartilage may here and there be seen.

The cartilage cells vary greatly in size and number, and may or may not have capsules. When pure, the chondromata are benign.

Osteoma.

An osteoma is a neoplasm consisting of bony tissue.

FIG. 79.—CHONDROMA. × 100.

Osteomata occur usually in connection with some portion of the skeleton as outgrowths (exostoses), and in the periosteum of bones, the tendons, and very rarely in the lungs, meninges, and skin.

In connection with sarcomatous tissue they are fre-

quent in the long bones and periosteum of the bones of the extremities and jaws, forming *osteosarcomata.*

Pure osteomata present microscopically the typical structure of normal bone and are benign growths.

Glioma.

A simple glioma is a neoplasm which has sprung from the neuroglia or nerve connective-tissue cells of the cen-

FIG. 80.—NEUROGLIOMA GANGLIONARE. × 160.

tral nervous structures. The gliomata follow the type of the neuroglia cells, and in section are seen to consist of a mass of cells with round or oval nuclei and possessing numerous protoplasmic processes whose delicate extremities interlace in a most complex manner, forming a rich network. These tumors may be very vascular and give

rise to severe hemorrhage. They may also become sarcomatous through proliferation of the cells of the perivascular connective tissue.

Those round-celled tumors of the eyeball which formerly were classed as gliomata are more properly round-celled sarcomata. They arise from the granular layer of the retina and usually run a very malignant course. The true gliomata are essentially benign.

A *neuroglioma ganglionare* consists of glia cells, large ganglionic nerve-cells, and nerve-fibers. These neoplasms are probably congenital.

Sarcoma.

A sarcoma may be said to be a malignant neoplasm arising from connective tissue, the cells being embryonic in type, without definite form of arrangement and bound together by a scanty intercellular matrix. They may or may not be encapsulated. The sarcomata always spring from previously existing connective tissue of some kind; the softer connective tissues occurring normally throughout the body or from the specialized connective tissues, bone, and cartilage. As connective tissue exists in one or another form in every portion of the animal body, it follows that sarcomata must have a wide field of origin. They occur more frequently in the dense structures, fasciæ, periosteum, skin, and bone, than in the internal organs, though they develop as primary growths in the uterus, kidney, liver, lungs, testes, and ovaries.

The varieties differ widely in the form of cells and amount of intercellular substance, and in the number and size of the blood-vessels.

The *soft* or *medullary sarcomata* are rich in cells, while the intercellular substance is scanty. The harder varieties are poor in cells and the intercellular substance is

composed of a dense fibrous tissue. These latter are *fibrosarcomata*.

The blood-vessels in sarcomata are usually atypical and poorly organized. Many of the smaller vessels may be composed of the cells of the tumor arranged in a more or less definite manner around the blood-channel, which has

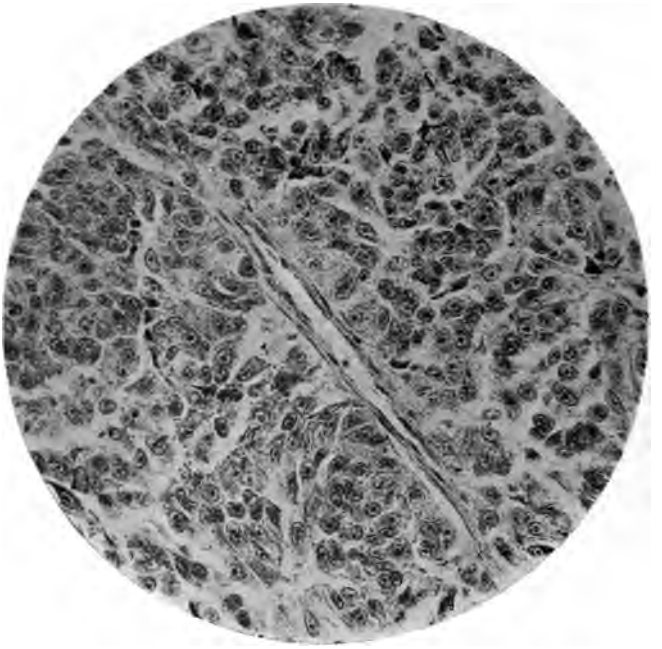

FIG. 81.—LARGE ROUND-CELLED SARCOMA.—(*Thayer.*)

the usual endothelial lining. In some sarcomata well-formed blood-vessels exist. The sarcomata appear to be devoid of lymph-vessels.

Degenerative changes are common in sarcomata, chief among these being fatty degeneration, necrosis, hemorrhage, gangrene, liquefaction, and ulceration.

Sarcomata are always malignant to a greater or less degree, though the encapsulated forms may be very slow in growth, and the fibroid changes may be such that the neoplasm becomes almost a pure fibroma. But even tumors of such a character form metastases which may prove of a malignant nature, or they may themselves take on a rapid and destructive growth.

According to the form of cell predominating, sarcomata are divided into:

Small round-celled sarcoma.
Large round-celled sarcoma.
Small spindle-celled sarcoma.
Large spindle-celled sarcoma.
Mixed sarcoma.

Small round-celled sarcoma is, as the name implies, composed chiefly of small round cells which have a large nucleus and a relatively small amount of protoplasm. The cells are held together by a scanty hyaline or fibrillated intercellular substance.

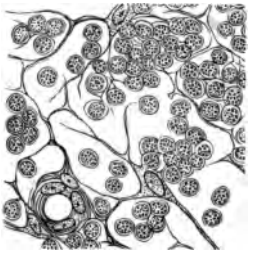

FIG. 82.— LYMPHOSARCOMA.—(*Gould.*)

The blood-vessels may be very numerous and vary in degree of organization, though generally they are little more than canals in the tumor-substance around which the tumor cells are more or less radially arranged.

The so-called *lymphosarcoma* belongs to the small round-celled variety, but differs from the above in that it has a well-formed reticulated ground-substance of connective tissue, many of the cells of which are branching. In this meshwork lie the tumor cells proper, and these may closely follow the type of cell found in the normal lymphatic gland. These latter tumors usually spring

from the various lymphatic tissues, but may occur in other situations.

The small round-celled sarcomata of either type are among the most malignant of neoplasms, as evinced by their rapid growth and frequent formation of metastases.

Large round-celled sarcomata differ from the small-celled variety in that they are composed of large cells having

FIG. 83.—SPINDLE-CELLED SARCOMA. × 360.

oval bladder-like nuclei and a large amount of protoplasm. They lie in a reticulum composed of branching connective-tissue cells with long anastomosing processes.

Some of the tumor cells possess more than one nucleus. The vessels are usually poorly developed. These tumors are usually not so malignant as the small-celled sarcomata.

Spindle-celled sarcomata are divided into the large and

small varieties, the one differing from the other only in the size of the cell, as either may be highly malignant or may follow a chronic or almost a benign course. They are composed of long spindle cells having large elongated nuclei and delicate protoplasmic prolongations at either end. Stellate cells may also be found.

The intercellular substance may be quite prominent or

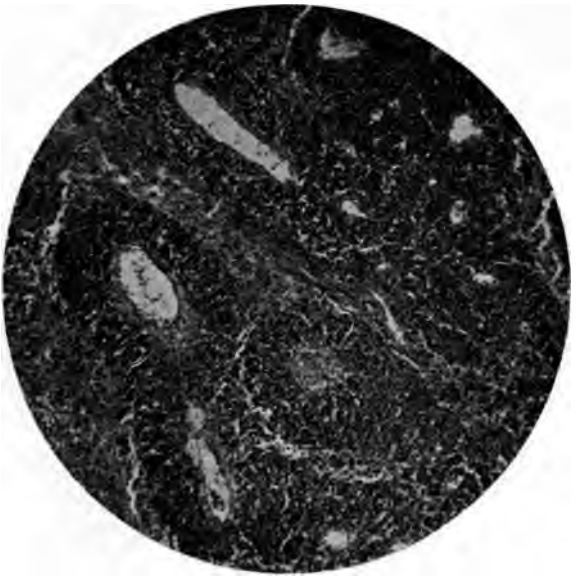

FIG. 84.—PERITHELIAL ANGIOSARCOMA. × 55.

so scanty as to be difficult of demonstration. The tumor cells frequently lie in bundles which may follow the course of the blood-vessels or arbitrarily cross each other in a complex manner.

Again, the cells may lie in a confused way entirely without perceptible motive. The fact that these growths may sometimes be confounded with leiomyomata has already been pointed out.

Mixed sarcomata are those in which there are both round and spindle cells in about equal amount.

Endotheliomata are those sarcomata which have sprung from endothelial cells. Such are not infrequently found arising from the lining of the blood- or lymph-vessels, or from the serous membranes of the body, pleura, and meninges. They form flattened growths which have all

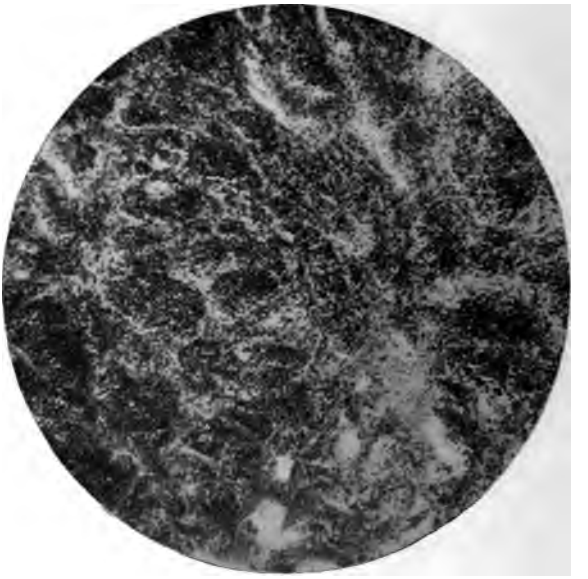

FIG. 85.—ALVEOLAR SARCOMA. × 100.

the characteristics of malignancy. Frequently the arrangement of the cells may be in tubular form, so that the glandular type of carcinoma is closely simulated. In such cases the origin of the tumor determines its true nature. The cells are frequently large and epithelioid in character.

Angiosarcomata are sarcomata growing from the walls of blood-vessels. The vessels may be surrounded by

dense deposits of cells forming columns, with dilated vessels as a core. The vessels may be very numerous and follow a complex course. To such growths the name *plexiform angiosarcoma* has been given. They have also been called *perithelial angiosarcoma.*

Alveolar sarcomata have a highly organized reticulum consisting of bands of connective tissue forming alveoli

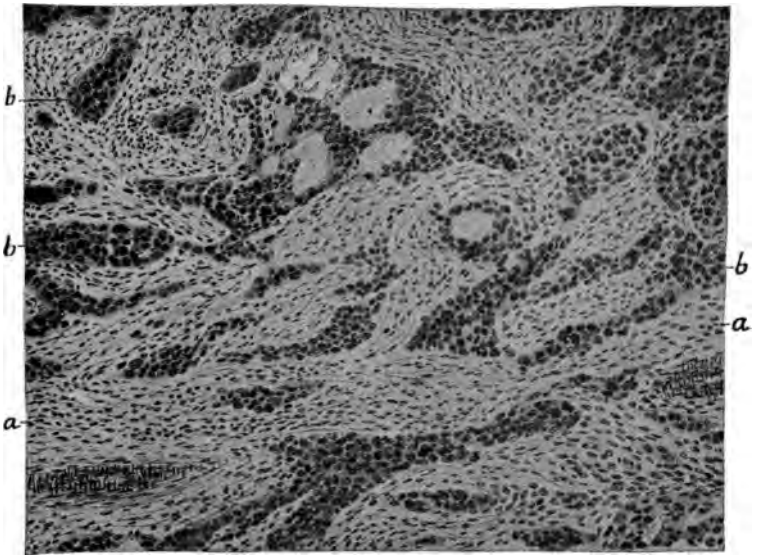

FIG. 86.—ENDOTHELIOMA OF PLEURA.—(*Ziegler.*)
a, Fibrous stroma. *b*, Cords of endothelial cells.

in which lie nests of sarcoma cells. The peculiar structure is probably formed by the tumor cells crowding in and pushing apart the normal connective tissue of the part.

Melanotic sarcomata may be of either variety described, but contain melanin or pigment in varying quantity. They are always extremely malignant. Their most frequent origin is the skin, pigmented moulds, or the

choroid of the eye. The pigment-granules are usually of a light yellow color.

Giant-celled sarcomata are frequently found arising from bone or periosteum. They are usually of the spindle-celled variety, but in addition have many large cells containing numerous nuclei. These nuclei may be quite separate and clearly outlined, or may be fused together in

FIG. 87.—MELANOTIC SARCOMA. × 55.

a poorly defined mass which may be to one side of the cell or may occupy its center. Because of the similarity of these cells to the myeloplaques of bone-marrow the tumor has received the name of *myeloid sarcoma.*

Such tumors are frequently slow in growth and do not form metastases, and therefore do little damage. On the other hand, they may be very malignant.

Not infrequently sarcomatous tissue is combined with a

varying quantity of some other tissue of the connective-tissue group. Thus, we have *myxosarcoma*, *chondrosarcoma*, *osteosarcoma*, and a variety of other combinations.

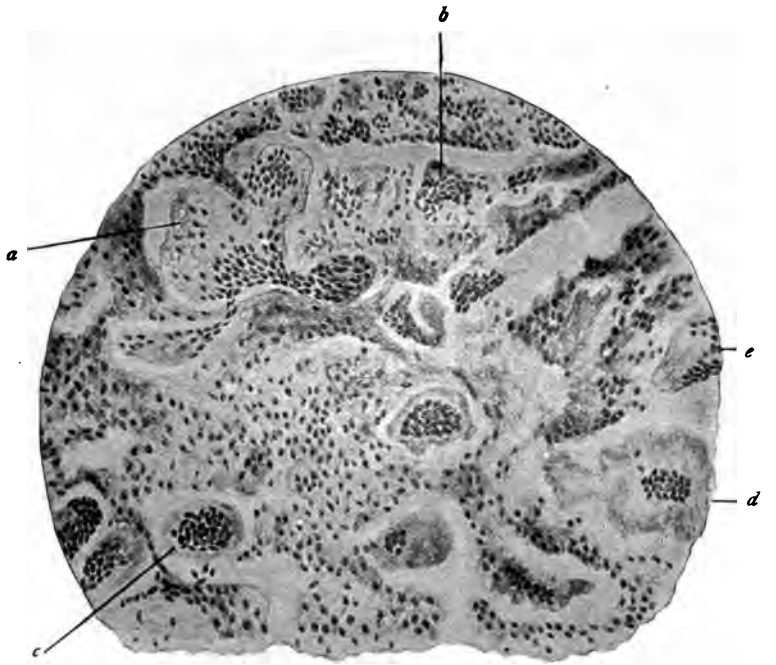

FIG. 88.—GIANT-CELLED SARCOMA.—(*Coplin.*)
a, *b*, *c*, *d*, *e*, Giant cells.

Secondary changes, softening, liquefaction necrosis, or calcification may take place in the sarcomata of either variety.

EPITHELIAL TUMORS.

The tumors about to be described differ from others in that they are derived from the epiblast and hypoblast, and are classed as epithelial growths.

The tumor cells proper are of the epithelial type of cells,

and they are separated by a connective-tissue framework of greater or less amount. The epithelial cells of the body normally go to make up those structures which are protective,—skin and mucous membranes,—and the secreting parts or glands.

We have seen that tumors follow more or less closely the normal structures from which they are developed, and so it is not surprising that we find the epithelial neoplasms made up of those tumors which are of a glandular type or which are similar in structure to the mucous membranes or are composed of cells arranged in a manner simulating the stratified epithelium of the skin.

Papillomata.

These neoplasms are *outgrowths* from the skin or mucous membranes. They usually arise from previously

FIG. 89.—PAPILLOMA.—(*Schmaus.*)

existing papillæ, and are by some authors considered hypoblastic growths rather than true neoplasms, or fibromata with an epithelial covering.

They consist of a central core of connective tissue, blood-vessels, nerves, and lymphatics, with a tunic of epithelial cells of the type normal to the part from whence the papilloma arises. The common *skin wart*, the soft *venereal wart*, and the *villous wart* of the bladder are common examples.

The papillomata are benign growths. They differ from the epitheliomata in that they grow *up* and not down into the tissues from which they arise, and present none of the other characteristics of malignancy.

They may produce death through mechanical means, hemorrhage, or obstruction in the respiratory or genito-urinary passages. They may also take on a malignant growth, becoming true epitheliomata.

Adenomata.

The **adenomata** are neoplasms which spring from and closely follow the type of some normal gland structure. They occur usually as round or pear-shaped tumors of comparatively slow growth, in the skin, where they arise from the sweat and sebaceous glands; in the mammary glands, uterus, liver, kidney, stomach, rectum, and other situations. They are comparatively rare growths, and when pure are benign.

It is, however, difficult and at times impossible to separate them, on the one hand, from simple inflammatory hyperplasia of a glandular structure, and, on the other, from the carcinomata. In making a diagnosis the verdict must be based upon the microscopic findings, together with the history and clinical symptoms of the individual case.

An adenoma differs from the normal gland structure in that it does not possess all of the anatomic elements, nor can it perform the physiologic functions of the gland from which it sprang and the type of which it follows. Ducts, if present, are abortive and end in a blind pouch.

Two chief forms of adenomata are recognized, one having a complicated arrangement of acini lined with columnar epithelium supported by a basement of connective tissue, the whole resembling a normal racemose gland. There may even be imperfectly developed excretory ducts. Such a growth is called an *alveolar adenoma.*

FIG. 90.—SIMPLE ADENOMA.—(*Coplin.*)

A simpler form is the *tubular adenoma.* As the name implies, this is made up of irregular, dilated tubules, composed of a connective-tissue framework, often quite dense, the spaces in which are lined by columnar epithelium. When either of the above by rapid growth of the epithelium forms finger-like or branching processes which pro-

ject into the lumen of the dilatations, it is known as a *papilliferous adenoma.*

Dependent upon the amount of connective tissue, an adenoma may be soft or hard.

When the epithelial cells of an adenoma develop a tendency to break out of the bounds set by the connective tissue upon which they rest, and to form new and independent nests of cells, the tumor may become destructive and form metastases, in which case it is called an *adenocarcinoma.*

Another form of adenocarcinoma consists of tubular structures similar to the original adenoma, malignancy being manifested by rapid growth, invasion of neighboring tissues, and metastases to more distant parts. The growth may retain its adenomatous type or may eventually become a true carcinoma. Such neoplasms are not uncommon in the stomach.

The adenomata are subject to secondary changes. They may become *cystic*, myxomatous, or even calcareous.

Carcinomata.

A carcinoma is a malignant neoplasm arising from epithelial structures and following the type of tissue from which it springs.

There are two great classes of epithelium, that which covers surfaces—skin, mucous membranes—and that which forms the secreting portions of glandular organs—mammary gland, liver, kidney, etc.

The first of these is composed of flat cells, usually in the stratified form. The second is composed of polyhedral cells in tubular or other glandular arrangement. It naturally follows that there are two great types of carcinoma—those arising from surface epithelium of the squamous type which follow this type of tissue and are

composed of *flat* cells, and those having their origin in glands and following the glandular arrangement of tissue more or less typically.

Epithelioma.—These neoplasms, as has been pointed out, arise from surfaces covered with stratified squamous epithelium. They are composed of columns of epithelial cells which penetrate the surrounding structures and

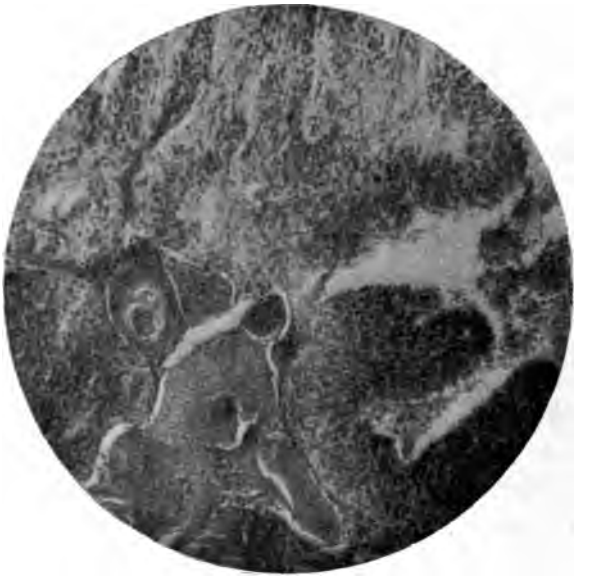

FIG. 91.—EPITHELIOMA. × 100.

which frequently form central whorls or nests of cells, the so-called *epithelial pearls*.

These nests are composed of concentrically arranged horny epithelium and represent an effort of the abnormally proliferating cells to conform to the normal type of stratified squamous epithelium.

It is safe to class the epitheliomata among the malig-

nant neoplasms, as they frequently grow with astonishing rapidity and cause great destruction of neighboring tissues and form metastases in distant parts. They are prone to degenerative processes, and frequently become infected with pyogenic organisms, resulting in ulceration and abscess.

Glandular Carcinoma.—In this form of cancer the tumor

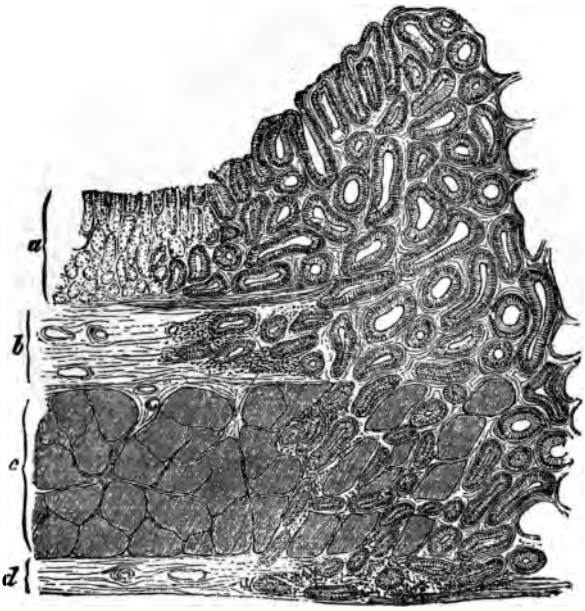

FIG. 92.—ADENOCARCINOMA OF STOMACH.—(*Ziegler.*)
a, Mucosa. *b*, Submucosa. *c*, Muscularis. *d*, Serosa. *e*, New growth proceeding from mucosa and infiltrating other layers.

is composed of a connective tissue in which lie groups of epithelial cells arranged in a manner more or less typical of normal glandular tissue.

The connective tissue does not penetrate between the individual cells, as in sarcoma, but surrounds *groups* of epithelial cells, so that if these epithelial aggregations

should be shaken out, a sponge-like mass of connective tissue would remain.

Adenocarcinoma, as has been shown, is but a step removed from true adenoma, and consists of a neoplastic tissue of distinctively glandular type the epithelial elements of which have a tendency to rapid proliferation, so

FIG. 93.—MELANOTIC CARCINOMA. × 100.

that they pile up one upon another, forming in some instances alveoli lined with two or more layers of cells. They also push out bud-like projections of epithelial cells into the neighboring tissues.

In the more common form of glandular carcinoma the alveoli are entirely filled by epithelial cells of a polyhedral type, so that no central lumen exists.

These growths are frequent in the mammary gland, liver, pancreas, intestines, and other glands.

Clinically they are divided into *medullary* or soft, *simple*, and *scirrhous* cancer. In the soft varieties there is a preponderance of the epithelial cells over the connective tissue, while in the hard or scirrhous cancers the opposite

FIG. 94.—MEDULLARY CARCINOMA. × 360.

condition prevails. The simple carcinoma is composed of about equal amounts of these two factors. This classification is of little value, as the same tumor may show all of the above varieties of structure in different parts.

Carcinoma myxomatoides is a form in which the connective-tissue framework has undergone mucoid changes.

Giant-celled carcinoma has large epithelial cells, many of

which may be dropsical. True giant cells with several nuclei may also be present.

Melanocarcinoma may be of any of the preceding forms, and is distinguished by the presence of pigment granules within the stroma and epithelial cells. This form is always very malignant.

FIG. 95.—SCIRRHOUS CARCINOMA. × 100.

Epithelial Cystomata.

From the simple retention cyst these tumors are distinguished by there being a distinct new-formation of tissue. They consist of one or more cavities containing a fluid or mucoid substance. The walls of the cavities are composed of connective tissue lined with epithelium, frequently of the columnar variety.

They cannot be sharply differentiated from either the

adenomata or carcinomata. Usually they are benign, but they may at any time take on a malignant character.

Papillary cystomata are distinguished by prolongations from the walls of the cysts, consisting of a connective-tissue core covered with one or more layers of epithelial cells. A rapidly growing tumor of this type constitutes the *papillary cystocarcinoma.* From such growths metas-

FIG. 96.—GIANT-CELLED CARCINOMA. × 360.

tases may occur that exhibit the characteristics of ordinary glandular carcinoma.

Teratomata.

A *teratoid tumor* is one of complex composition, and "consists, at least in part, of tissues which do not nor-

mally occur at the site where the tumor is found" (Ziegler).

The *dermoid cyst* belongs to this class. The rhabdomyomata of the kidney may also be cited as an example,

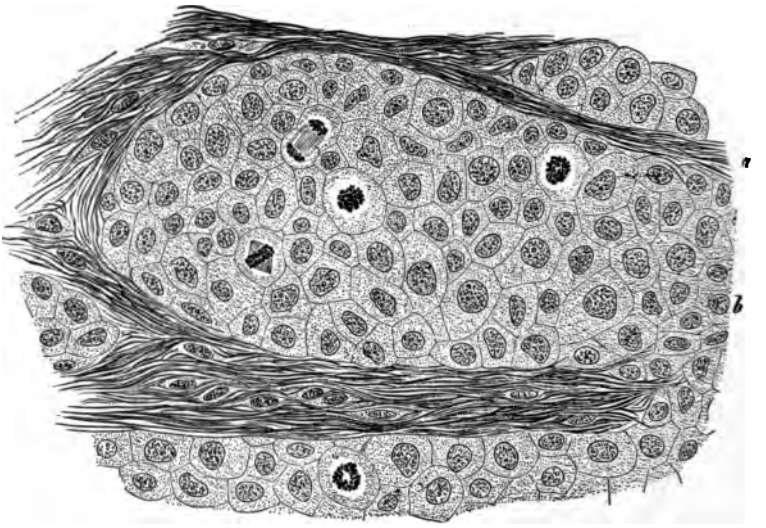

FIG. 97.—SIMPLE CARCINOMA.

a, Fibrous trabeculær. *b*, Large atypical epithelia of polygonal outline, showing mitotic figures.—(*Zeigler.*)

and rarely there are retroperitoneal neoplasms presenting structures similar to those of the gastro-intestinal tract.

The teratomata are usually benign, but may become sarcomatous. They are supposed to be congenital.

THE BLOOD.

Histology of the Blood.

The blood consists of a fluid *plasma* in which float red and colorless blood-corpuscles and blood-platelets.

The normal *blood-plasma* is a thin, colorless fluid of alkaline reaction.

The *red blood-cells* of normal adult human blood are biconcave discs about 7.5 μ in diameter. They consist of semifluid protoplasm and a coloring-matter, the *hemoglobin*, upon which the staining quality of the cell directly depends.

The question as to whether they have a definite cell-wall still remains unsettled. Probably there is such a structure, or it may be that the appearance of one is given by the condensation of the protoplasm of the cell about its periphery. The red cells are capable of great mobility and in fresh blood or in the capillary vessels, as seen in the web of a frog's foot, they readily assume various shapes, accommodating themselves to the caliber of the channel, or are indented by contact with neighboring cells. They immediately assume their normal form when the pressure is removed. Frequently they collect in coin-like masses or *rouleaux*.

The non-nucleated, biconcave form of red blood-corpuscle is found in all mammals with the exception of the camel, in which the red blood-cells are *oval*, but contain no nucleus.

Oval nucleated red corpuscles are normally in the blood of all fowls, reptiles, and fishes, with the exception of the *Cyclostomata*, in which the cells are round, discoidal, and nucleated.

The largest of all red blood-corpuscles are those of the

Amphiuma, which are about 0.046 mm. wide and 0.075 mm. long, and may be seen by the unaided eye.

Development of the Red Blood-cells.

In early fetal life the development of the red blood-corpuscles is thoroughly determined, while their origin in the latter part of fetal existence and during adult life is still a matter of uncertainty.

The red cells first appear in early embryonic life as isolated groups of cells in the so-called "islands of Pander" or vascular areas. They are of mesodermal origin and are formed simultaneously with the first blood-vessels and from the same group of cells. At a later stage the liver and lymphatic glands contribute largely toward the manufacture of red blood-cells. After the formation of the bones the red marrow plays an important part in this function, and is considered by most authors to be the exclusive site of the manufacture of the red blood-cell during extra-uterine life, though by some it is held that under pathologic conditions the spleen may, as it were, return to a fetal condition and again take up the labor of producing red blood-corpuscles.

Varieties of Red Blood-corpuscles.

The normal red blood-cell or erythrocyte of adult life has been described. There are, however, in embryonic life and pathologic conditions other forms of red blood-corpuscles that now deserve our attention. They are classified according to size into:

NUCLEATED.	NON-NUCLEATED.
Microblasts.	Microcytes.
Normoblasts.	Normocytes (Erythrocytes).
Megaloblasts.	Megalocytes.

All varieties of red blood-cells at first contain a nucleus which at a later stage disappears entirely or is demonstrable only in part and with difficulty. The nucleus in most cases probably disintegrates and fades *in situ*, though Ehrlich and Lazarus contend that this is not the case with the nucleus of the normoblast, but that it is extruded from the cell entire. As free nuclei of red blood-cells may frequently be met with, this view is evidently, at least in part, correct.

From the above tabulation it will be seen that when the red blood-cell loses its nucleus it takes the termination *cyte*, so that upon the extrusion of the nucleus of a normo*blast* it becomes a normo*cyte* (erythrocyte) or normal red blood-corpuscle of adult life.

Microblasts.—These are red blood-cells that are considerably smaller than the erythrocyte and which measure from 1 μ to 4 μ in diameter. The nucleus is single and stains very deeply with all nuclear dyes. It may be proportionally very large, there being only a small rim of protoplasm present.

They probably arise from division of degenerated or immature mother-cells, and are not uncommon in pernicious anemia and splenic myelogenous leukemia. They are also frequent in the fetal blood.

Microcytes are small *non-nucleated* red blood-cells derived either from the microblasts or by the contraction of erythrocytes due to loss of hemoglobin or the fragmentation of degenerating red cells of normal size. They are common in blood that is undergoing severe and rapid degenerative changes. Usually they are deficient in hemoglobin, and therefore do not take up dyes readily and appear pale and thin.

Normoblasts are the progenitors of the erythrocytes, and are therefore about 7.5 μ in diameter. The nucleus

is usually single, occupies about one-third of the corpuscle, and stains more deeply than the nucleus of any other cell. The protoplasm is rich in hemoglobin, and therefore takes the dye with avidity, being deeper in color than in the erythrocytes. Normoblasts are found in fetal life, and even in small quantity for some time after birth in normal blood.

Normocytes (**erythrocytes**) have been already described; they constitute the normal red blood-corpuscle of adult life.

Megaloblasts.

These are nucleated red blood-cells and are from 10 μ to 20 μ in diameter. The larger forms are sometimes called *gigantoblasts*. They differ from normoblasts in several important particulars. Besides their large size, the nucleus is frequently multiple, lobulated, or stellate, or may show various stages of mitosis, usually of an imperfect character. Generally there is to be distinguished about the nucleus a clear zone which evidently is devoid of hemoglobin and therefore does not take the stain.

The nucleus is deeply though often irregularly stained, and may vary in color in different parts owing to degenerative changes. The protoplasm usually stains deeply, but may show a loss of hemoglobin and corresponding paleness, and at times there may be granular fragments of the nucleus in the protoplasm. When found in considerable numbers, they always indicate pernicious anemia of the primary type, but a few may occur in secondary anemias. They are also present in leukemia, often in large amount.

Megalocytes are derived from the megaloblasts by degeneration of the nucleus of the latter. They are from 10 μ to 20 μ in diameter and may show either an excess

or a deficiency of hemoglobin. At times they may undoubtedly be formed by the swelling of the normocytes due to altered conditions of the blood-plasma, as may be seen in cases of severe malaria. Usually they are accompanied by megaloblasts, and are therefore chiefly found in those conditions in which the latter obtain.

Blood-plates.

The origin and physiology of these bodies is not yet determined. They occur in normal blood in considerable numbers as oval or spheroidal bodies from 1 μ to 3 μ in diameter, and stain faintly with methylene-blue and other basic dyes. They have a marked tendency to form in clumps, and may then appear as a mass the individual components of which it is not possible to make out.

The author has recently observed them in specimens stained by Goldhorn's method apparently lying *within* the red blood-corpuscle, though possibly this is an artifact. In such cases, however, the most careful focusing fails to show that they lie at a level different from that occupied by the red blood-cell, nor is its characteristic coloration seen through them. With the above stain they appear, when discrete, as round bodies, having a clear-cut outline of colorless protoplasm (?) in which is a recticular structure of a brownish-red color. The stain of this reticulum is exactly similar to the chromatin of the malarial parasite as dyed by the Goldhorn method. This would seem to indicate that they are derived from nuclear elements, as has been frequently contended by various observers who consider them to be the results of fragmentation of the nuclei of degenerating leukocytes.

Blood-dust.—This name was given by H. F. Müller to granular colorless particles first observed by him in fresh blood. They are highly refractile and have a rapid danc-

ing (Brownian) movement. Their significance is not known.

WHITE BLOOD-CORPUSCLES OF NORMAL BLOOD.

The classification of the leukocytes is based upon their morphology and their reaction to certain staining agents of complex nature, for it is found that the protoplasm of some forms of white blood-cells contains granules differing in their action toward dyes from the granules of other leukocytes which, except in this respect, are identical.

Three forms of granules are distinguishable:

1. Basophile, ...Staining with purely basic dyes—methylene-blue, hematoxylin, thionin, dahlia, etc.
2. Oxyphile or Acidophile, ..Staining with purely acid dyes—eosin, fuchsin, etc.
3. Neutrophile, ...Staining with combinations of basic and acid dyes, forming a new specific dye. Ehrlich's tri-acid, Jenner's stain, etc. These latter combinations stain also one or both of the other varieties of granules.

Ehrlich's tri-acid stain is the one generally employed for the purpose of differentiating the white blood-cells. This has the property of staining two kinds of granules, the neutrophilic and the oxyphilic, but does not stain the basophile granules.

In order to demonstrate these latter we must use either a purely basic dye or a combination of pigments, such as Jenner's stain furnishes, which has the property of staining each of the three kinds of granules.

Lymphocytes.

These cells constitute about 22 to 25 per cent. of the white blood-corpuscles of normal blood. They are about

the size of a red blood-corpuscle and possess a central round nucleus the reticulated structure of which is often visible in well-stained specimens. The protoplasm is of comparatively small amount and is strongly basophilic, and in many instances is more deeply stained than the nucleus itself. The protoplasm is reticulated, as may be seen by its unequal staining properties in different parts. Frequently portions of the protoplasm may bud off and become separated from the mother-cell and float free in the blood. At times larger forms of lymphocytes are to be found. Their recognition is easy on account of the strongly basophilic protoplasm and the absence of proper granulations. Occasionally may be seen lymphocytes having notched or double nuclei. The lymphocytes stain more deeply than other cells found in the blood with the exception of normoblasts, from which they are readily distinguished.

Large Mononuclear Leukocytes.—In normal blood these are found in small numbers, forming only about one per cent. of the total amount of white blood-corpuscles. They are quite unique and easily recognizable on account of their large size, two or three times that of the erythrocytes, their large, pale, oval nucleus, and faintly basophilic protoplasm, which is devoid of proper granules. The protoplasm is finely reticular and abundant, stains less deeply than the nucleus, and in it the nucleus is generally excentrically placed.

Except in deeply stained specimens it is easy for the beginner to overlook these cells, on account of their universal paleness, which contrasts so greatly with the character of the other forms of leukocytes. Nucleoli are sometimes demonstrable.

Transitional Forms.—These are similar to and represent a later stage of the development of the large mononuclear

leukocyte. They differ from them in that the nucleus stains slightly deeper and shows a distinct notch in one side, giving it a horseshoe shape. There may be a few small neutrophilic granules in the protoplasm. Together with the large mononuclear forms, these constitute from 2 to 4 per cent. of the white blood-cells of normal blood.

Polynuclear Leukocytes.—These may be considered the most important of the white blood-cells, constituting as they do 70 to 72 per cent. of the total amount of these elements in normal blood. They are usually about twice the size of the erythrocyte. The nuclei show a great variety of forms, being frequently Z-, S-, or E-shaped, the various portions connected by delicate threads (polymorphonuclear), or there may be several distinct nuclei, the latter fact having led Ehrlich to give them the above not strictly descriptive name.

The nucleus is colored deeply with all nuclear stains and exhibits a coarse recticulum. The protoplasm is finely reticulated, slightly basophilic, and contains numerous neutrophilic granules, of varying size but usually quite fine.

Eosinophiles.

These are polynuclear cells about the size of a polynuclear leukocyte, though they may be somewhat smaller. The nucleus is coarsely reticular and stains less deeply with nuclear stains than does that of the polynuclear leukocyte.

Their distinguishing feature is the large deeply staining oxyphilic granules of the protoplasm. With eosin these appear as numerous shot-like bodies of a deep red color, while with Ehrlich's tri-acid stain they are a rich copper color. In fresh blood on the warm stage, or even at room-temperature, they are exceedingly active, often traveling

by ameboid movement entirely across the field, teh streaming motion of the granules being distinctly visible. *In fresh preparations the beginner often mistakes them for the malarial parasite—an error to be guarded against.*

They constitute about 2 to 4 per cent. of the white cells.

TABULATION OF CELLS ACCORDING TO STAINING REACTION.

1. *Basophile Cells.*
   Lymphocytes. Protoplasm strongly basophilic.
   Large mononuclear leukocytes. Protoplasm faintly basophilic.
   Mast Cells. Granules strongly basophilic.
2. *Oxyphile Cells.*
   Polynuclear Eosinophiles. Granules oxyphilic.
   Eosinophilic Myelocytes. Granules oxyphilic.
3. *Neutrophile Cells.*
   Polynuclear Leukocytes. Granules neutrophilic
   Myelocytes. Granules neutrophilic.

Nature of the Granules.

As to the exact structure and significance of the granules of the white blood-corpuscle there has been considerable controversy. According to recent views, "they are a secretory product, and represent the specific function of the cell" (Ewing).

It has also of late been shown that the basophilic cells are possessed of a reticulate structure which is continuous with the spongioplasm of the nucleus. Where the lines of this fine network connect with each other there are found nodal thickenings. It has been demonstrated that the granulations of the cells are connected with this reticulum by fine lines, and they are therefore integral parts of it.

Nucleoli may also be demonstrated in the nuclei of the leukocytes.

Mast Cells.

These must be considered as rather accidental in normal blood, where they are rarely found; according to

Ehrlich, they are present in the proportion of 0.5 per cent. of white cells.

In size and in the form of the nucleus they are very irregular, but from other cells they are sharply defined by large granules in the protoplasm which stain deeply with basic dyes. With certain stains, notably thionin and polychrome methylene-blue, these granules do not show the pure color of the dye, but stain metachromatically.

The nuclei do not stain deeply, and it must be remembered that with Ehrlich's tri-acid stain the granules are unstained, so that the protoplasm of the cell remains clear.

COLORLESS CELLS PECULIAR TO PATHOLOGIC CONDITIONS OF THE BLOOD.

Myelocytes.

These vary in size from about that of an erythrocyte to several times this diameter. They have a large, round or oval, pale-staining nucleus and a relatively large amount of protoplasm, and may be easily distinguished from the large mononuclear leukocyte, which they otherwise resemble, by the numerous neutrophilic granulations of their protoplasm.

They are never present in normal blood. In myelogenic leukemia they may occur in enormous numbers, and they have been observed in smaller quantities in various infectious diseases, in poisoning by mercury, in the anemia of infantile pseudoleukemia, and in diphtheria and pneumonia.

Eosinophilic Myelocytes.

In myelogenic leukemia and infantile pseudoleukemia mononuclear cells with oxyphilic granulations of the pro-

toplasm are frequent, but they rarely occur in other conditions.

Small Neutrophile Pseudo-lymphocytes.

Ehrlich describes these cells, which he first found in a case of hemorrhagic smallpox, and considers them as products of division of the polynuclear cells. They are about the size of a lymphocyte and present a small amount of protoplasm studded with neutrophile granules.

TECHNIQUE.

So delicate a structure as a blood-cell requires faultless technique if we would see it in its normal state.

The beginner must first perfect himself in the various methods, using his own or other normal blood, before he ventures to draw conclusions from blood derived from presumably diseased subjects. Not one but many preparations should be made and studied, so that he will become thoroughly familiar with the normal histology of the blood and with those artifacts that in spite of all caution occur in preparations made by the most experienced workers. The study of the blood is so fascinating that such a labor is light.

Except in the case of malaria it is rarely advisable to study blood in the fresh, unstained condition. A description of the method of making fresh preparations is given in the section on malaria. For most purposes we resort to thin spreads of blood that have been stained by some dye.

A close attention to minute details is necessary to insure success. It is quite necessary for the student to recognize this in the beginning, as it will save him vexatious failures.

Making the Smear.

Many text-books recommend the use of cover-glasses for this purpose. The author much prefers to use the glass slip, for the reasons that it is easier to handle, the smear is more apt to be successful, it can be carried through the various manipulations with greater ease and certainty, and it is better *not* to use balsam and a cover-glass upon the finished product, as the preparation is apt to fade, while it keeps perfectly for years when left uncovered.

The slips must be absolutely clean in order to insure thin

FIG. 98.—HOW TO MAKE A BLOOD-SMEAR.

and even smears. It is well to first clean them with alcohol and a clean cloth, rubbing them vigorously. They should be again briskly rubbed with a clean cloth immediately before use, for the double purpose of removing any dust that may have settled on them and of warming the glass by the friction, as this facilitates the spreading of the blood.

The lobe of the ear is the best place from which to obtain the drop of blood. In order to rid the skin of grease and dirt and free epithelial scales the part should be cleaned with a clean cloth, soap and water. It is entirely

unnecessary to use alcohol and ether, as is frequently recommended.

For the purpose of making the puncture a small lance, such as is sold for the purpose, or a thin-bladed tenotome should be used. The instrument should be very sharp, as it will give less pain to the patient and produce a better flow of blood. After use it should be washed and wiped clean. Experience shows that it is not necessary to sterilize it except in cases known to be septic. The lobe of

FIG. 99.—HOW NOT TO MAKE A BLOOD-SMEAR.

the ear is held between the thumb and finger of one hand and strong pressure gradually made upon it for a moment and the point of the lance then thrust with a quick movement a sufficient depth into the tissue. In nervous patients and in children by following this method it will be found that frequently the puncture may be made without the knowledge of the patient.

The puncture should be deep enough to allow the blood to flow spontaneously or with a gentle "milking" pressure. The first few drops are to be wiped away.

A cleaned slip is laid upon a convenient table; another, held by the end between the thumb and finger of the right hand, is to be used to make the smear. Milk out a fresh drop of blood; bring the free end of the slip in contact with it so that a small drop will cling to its *edge;* quickly transfer this to the slip upon the table by allowing the end of the slip containing the blood-drop to come in contact with its surface a short distance from its further end. The upper slip is now dragged along the surface of the lower one, the blood-drop gradually escaping beneath it and being distributed in a thin, even smear.

In this latter part of the manipulation the slip must not be held at too great an angle. The proper angle is best determined by allowing the free end of the slip to lie upon the tip of the index-finger, which is then drawn along the surface of the table, the weight of the slip being sufficient to carry it along at the same time. A much better result is thus obtained than if the slip is grasped firmly between the thumb and finger, as a steady movement is then impossible and an unequal smear results.

Caution: The drop of blood must be small; a large drop means a thick, useless smear.

The smear is allowed to dry in the air, which it does very quickly, and the slip may now be wrapped in paper and carried to the laboratory.

Fixation.

If Ehrlich's tri-acid stain is to be employed, it is necessary to fix the blood by *heat.* This is best accomplished by the use of the hot-air sterilizer, or some other form of oven in which a high degree of dry heat can be obtained.

The author secures the best results by putting the smears in the oven when it is cold and gradually raising the temperature to 125° C., at which point it is kept from

ten to fifteen minutes. The smears are now removed *and cooled* before applying the stain.

Fixation by heat may be accomplished by passing the slip repeatedly through the bunsen flame until it is too hot to be borne in contact with the hand. This degree of heat must be kept up for about five minutes. After experience it is possible to obtain fairly uniform results in this way, but the method is at best a makeshift and unreliable.

For methylene-blue, eosin, and other stains it is not necessary to use heat as a fixing agent. Either of the following methods is good:

1. Immerse the slide for at least fifteen seconds in methyl alcohol.
2. Wash in water.

MODIFICATION OF FUTCHER'S METHOD.

1. Formalin (full strength), ... 2 drops
   95 per cent. alcohol, ...10 c.c.

Immerse the smears for at least one minute.

2. Wash in water.

This solution must be freshly made.

Jenner's stain fixes and stains at the same time on account of the dye being dissolved in methyl alcohol.

STAINS.

Ehrlich's Tri-acid Stain.

This is a complex stain and is very difficult to make successfully. Reliable solutions may be obtained from various dealers and the formula for the above reasons is not given.

With a glass rod a drop of the stain is evenly spread over the smear previously fixed by heat, as above indicated.

The stain should be allowed to act for three minutes or longer. It is impossible to overstain with this mixture, and if the solution dries upon the smear no harm results.

Wash in running water until no more stain flows from the preparation, blot, dry, and examine with the $\frac{1}{12}$ objective without the use of a cover-glass, the drop of oil being placed directly upon the smear.

If the method has been properly carried out, the result will be as follows:

Erythrocytes: golden yellow.
Nucleated red blood-cells: nuclei dark purplish-brown, protoplasm brownish.
Lymphocytes: nuclei pale blue, protoplasm deeper blue.
Large mononuclear leukocytes: nuclei pale blue, protoplasm, faint pink.
Polynuclear leukocytes: nuclei robin's-egg blue, neutrophilic granules purple.
Eosinophiles: nuclei pale blue, oxyphilic granules large, round, and copper-colored.

Neutrophilic and oxyphilic granules in other cells as indicated above.

Basophilic granules are not stained.

If the smear is underheated, the stain is diffuse, definition poor, and the erythrocytes dark reddish-brown.

If the smear is overheated, the nuclei are pale, fragmented, and brownish, and the erythrocytes pale yellow.

Methylene-blue and Eosin.

For ordinary purposes where an exact differentiation of the varieties of colorless cells is not desired this stain is very serviceable. Its exact method of application is described in the technique for malarial blood smears.

Jenner's Stain.

The dry stain may be had from the shops, or may be made as follows:

Mix equal parts of a 1.20 to 1.25 aqueous solution of yellow water-soluble eosin and a 1 per cent. aqueous solution of methylene-blue and allow them to stand in an open vessel for twenty-four hours. Filter and dry the filtrate, which is then to be collected, powdered, and mixed with a quantity of distilled water and again filtered out. The resulting filtrate is powdered and may be kept in a well-stoppered bottle for future use.

To make the stain dissolve 0.5 gram of this powder in 100 c.c. of rectified methyl alcohol.

In this solution smears are to be stained from two to three minutes. It will be found that the best results will be obtained by immersing the smears in a Stender jar containing sufficient fluid for the purpose, and it is best to time them by the watch, regulating the time according to the action of the particular stain in use, as in the author's experience the action of this stain is not always constant in different samples. It is, however, an excellent method, staining, as it does, all forms of granulations and also the malarial organism.

If the nuclei are not well stained by this method it may be on account of excessive acidity, and a few drops of a dilute solution of caustic potash should be added to the stain.

The results are more accurate with freshly prepared specimens—not more than several days old.

Care must be taken to keep the jar of stain well stoppered, as on account of its alcoholic nature the stain rapidly evaporates. Simon recommends the addition of 10 per cent. glycerin.

By this method all nuclei are stained blue, the neutro-

phile granules reddish-purple, oxyphile granules bright red, and basophile granules deep violet.

The stain is also suitable for the malarial organism, which is stained a brilliant blue.

Differential Blood-counts.

It is frequently of the utmost importance to ascertain the relative proportions of white blood-corpuscles. This is accomplished by counting several hundred in a preparation stained either by Ehrlich's or Jenner's method, the number of each variety being noted. It is then a simple matter to calculate the relative percentage of each kind of leukocyte present.

In order that we may not fall into error by seeing the same cell a number of times, a mechanical stage is useful for this work, as it insures a uniform and accurate movement over the whole surface of the preparation; neither must it be lost sight of that in the most carefully prepared specimens the leukocytes are prone to aggregate at the *edges* of the smear in groups. This is probably due to their being larger than the red cells, so that they cling longer to the slip with which the smear is made.

NUMBER OF BLOOD-CORPUSCLES.

The number of both the red and white corpuscles is subject to some variation within physiologic limits, and in pathologic conditions these variations may be extreme.

In a condition of health the number of red blood-corpuscles approximates 5,000,000 per cubic millimeter in the adult male, the number for the adult female being generally given at 4,500,000 per cubic millimeter.

The white blood-corpuscles are much more liable to wide variations within physiologic bounds than are the

red cells. They vary according to the age, sex, and general habit of the particular individual, the time of day, during pregnancy and digestion, and we must therefore fix boundaries not too near together in determining the normal numbers of these constituents of the blood. It is safe to consider that a fall below 3000 and a rise above 10,000 white blood-cells in the cubic millimeter of blood is abnormal, provided the variation is in any degree constant.

Thayer has noted a marked increase (*hyperleukocytosis*) in his own blood following a cold bath, the leukocytes before the bath numbering 3250, while twenty minutes after the bath they were increased to 13,170 per cubic millimeter. A notable increase has also been observed in typhoid patients after receiving the Brand bath.

ENUMERATION OF RED BLOOD-CORPUSCLES.

The determination of the number of red blood-cells per cubic millimeter is often of the greatest interest and value.

FIG. 100.—THOMA-ZEISS HEMOCYTOMETER.

Among the instruments devised for this purpose the hemocytometer of Thoma stands first. The one made by the firm of Carl Zeiss is usually employed, though the

Reichert instrument is also reliable. The parts of the instrument as shown in the cut (Fig. 101) consist of a glass slip containing a cell of known diameter the bottom of

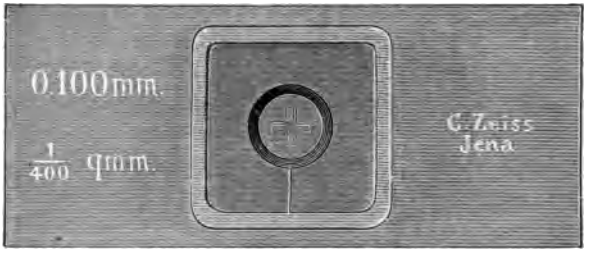

FIG. 101.—BLOOD-COUNTING CHAMBER.

which is ruled as shown in figure 102, the heavier lines embracing blocks of twenty-five smaller squares. A cover-

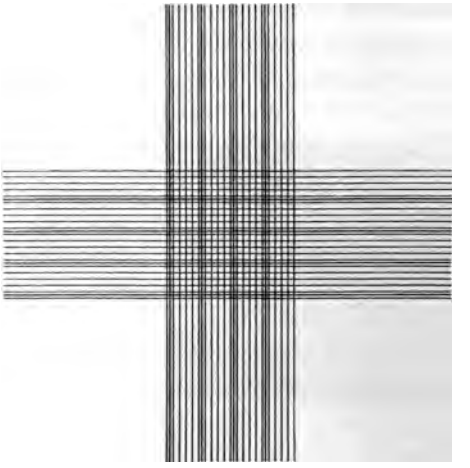

FIG. 102.—RULINGS OF HEMOCYTOMETER.

glass with an optically true surface is provided, and this when placed on the slide forms a closed chamber exactly

0.1 mm. in depth, so that each of the small squares, of which there are 400, represents 0.0025 sq. mm.

For the proper dilution of the blood there is provided a glass pipette having at its upper portion a bulbous enlargement containing a small glass ball. The pipette is divided into parts of 0.1 cubic millimeter, while the bulb holds exactly 100 cubic millimeters, so that when the tube is filled to the 1 mark with blood, and the proper diluting fluid is then drawn in until the point marked 101 is reached, a dilution of the blood in the proportion of 1 : 100 is formed.

TECHNIQUE OF COUNTING RED BLOOD-CELLS.

Diluting Fluids.

For this purpose one may use a simple normal salt solution (0.6 sodium chlorid in water) or the following:

Hayem's Fluid:

Bichlorid of mercury,	0.5 gm.
Sodium sulphate,	5 0 gm.
Sodium chlorid,	1.0 gm.
Distilled water,	200.0 gm.

The drop of blood may be obtained either from the finger or ear of the patient, or, in the case of infants, from the great toe. The part must be thoroughly cleaned with soap and water, alcohol, and ether, and a free incision made with a suitable instrument so that the blood will flow of its own accord or with very slight pressure.

It must be seen that the circulation of the part is active, and, in a word, as nearly normal as possible to secure it, otherwise the count resulting will be subject to error.

The first few drops are to be wiped away and the capillary tube carefully filled exactly to the 1 mark and the

blood adhering to its point immediately removed with a clean cloth. The end of the pipette is now inserted in the diluting fluid and suction made until the tube is filled to the 101 mark. The pipette is now agitated to mix the blood thoroughly with the diluent.

The counting chamber is washed out with a little water and thoroughly dried and the cover-glass cleaned of dust.

Some of the fluid in the pipette is now expelled to get rid of that part in the capillary portion which consists of the diluent alone, and a small drop of the mixture is placed in the center of the ruled platform of the slide. The size of this drop can be determined by experience alone, and often several attempts must be made before the exact amount necessary is secured.

The cover-glass is grasped with a cover-glass forceps, its under surface is breathed upon, and it is then quickly lowered in place upon the cell. If the proper sized drop has been expelled and there are no dust particles present, the fluid will spread evenly between the two surfaces and *exactly* fill the space between them, none escaping into the well about the raised platform.

The cover-glass is to be gently pressed upon the slide with the butt of the forceps, the pressure being made upon its *sides* rather than in the center.

The whole procedure must be rapidly carried out, and if successfully accomplished a series of colored rings—Newton's rings—will appear beneath the cover, and on holding the preparation over a piece of white paper it will be seen that the blood is distributed in an even layer over the whole extent of the ruled surface.

Should these conditions not be secured, or if there are any bubbles present, a new specimen must be made. The specimen must be allowed to stand for a few moments so that the blood-cells may settle.

Counting.

The $\frac{1}{8}$ objective is to be employed for this purpose, a subdued light being used, as by it the blood-cells are brought out with greater distinctness. The lower left-hand block of twenty-five squares is brought into the field and the red blood-cells in each small square, beginning with the left upper square, are counted.

As many of the cells will fall upon the dividing-line, in

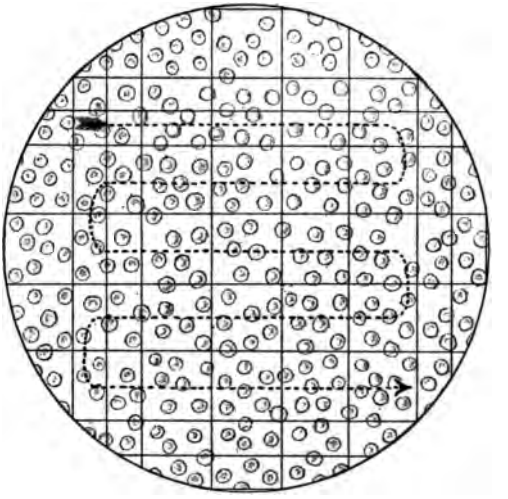

FIG. 103.—PLAN OF COUNTING ERYTHROCYTES.

order to avoid error one must count as in the square under observation all those upon the left-hand and bottom lines, leaving those on the lines to the right, which will by this method be counted in the next square. It is well to make a rough ruling on paper of lines forming a block of 25 squares and as each square is counted the result can be set down in the corresponding square of the diagram.

At least four of the blocks of 25 squares must be

counted, and the result is more accurate if twice this number be enumerated.

Computation.

If now we have as a result of counting the cells in 100 of the small squares a total of 1250 red cells, we will obtain the number of red cells in a cubic millimeter of blood by multiplying the product of the count by 4, for we have counted one-fourth of the surface of the square millimeter; again by 10, because the cell is only $\frac{1}{10}$ mm. deep; and again by 100, on account of the blood having been diluted with 100 parts of fluid. We have, therefore, $1250 \times 4 \times 10 \times 100 = 5{,}000{,}000$ per cubic millimeter.

As $4 \times 10 \times 100 = 4000$, when we have counted 100 of the small squares the multiplier is 4000; if 200 squares, 2000; and if 400 squares, 1000.

ENUMERATION OF WHITE BLOOD-CELLS.

The normal number of white blood-cells being so small, it is usually necessary for this purpose to have a special pipette, which allows of a dilution of 1 : 10 or 1 : 20.

In order to count the leukocytes we must get rid of the red blood-cells, as otherwise these would obscure the white cells. This is accomplished by using as a diluent a solution of 3 per cent. acetic acid slightly tinted with gentian-violet, which rapidly decolorizes all of the red blood-corpuscles and also renders the leukocytes more plain by staining them a pale violet color.

Otherwise than above, the technique of preparation of the drop is the same as that for the red-colored corpuscle.

The lower power can be used for counting leukocytes, and the contents of each of the small squares of the square millimeter should be enumerated.

If the dilution has been 1 : 10, the sum total is multiplied by 100, the result being the number of leukocytes per cubic millimeter.

Care of the Hemocytometer.

The greatest cleanliness must be observed in order to keep the apparatus in proper condition.

After using, the cell and cover should be washed with *water*—not ether or alcohol, as these will dissolve the

FIG. 104.—GOWERS' HEMOGLOBINOMETER.

cement with which the cell is attached to the slide—and dried with a clean soft cloth.

The pipette is cleaned by first sucking water into it, then with alcohol, and lastly with pure ether. This must *always* be done *immediately* after use. It is well occasionally to clean the pipette with pure nitric acid followed with alcohol and ether.

Estimation of Hemoglobin.

Various instruments have been invented for the purpose of determining the percentage of hemoglobin, none of

which is entirely satisfactory. Only the simple modifications of Gowers' hemoglobinometer will be described, as this is largely used, and for clinical purposes answers fairly well, as a slight error is of little consequence.

When the percentage of hemoglobin is known to be very low, twice the quantity of blood should be employed and a corresponding division of the result made.

The apparatus is exceedingly simple. It consists of a tube containing a standard solution of the tint of normal blood diluted with 100 parts of water, a second tube graduated to 120 parts, and a capillary pipette with which to measure 20 c.c. of blood.

Technique.

The tubes are placed side by side in the stand provided for them and a few drops of distilled water are placed in the tube in which the dilution is to be made.

The blood is obtained from a free puncture in the lobe of the ear or finger, the part having previously been cleaned with soap and water. The pipette is filled to the 20 c.c. mark and the contents are quickly discharged into the graduated tube.

The pipette is several times filled with distilled water and emptied into the graduated tube. The blood and water are mixed by agitation, and more water added drop by drop until the color of the mixture exactly corresponds with that of the standard tint tube.

The percentage of hemoglobin corresponds to the height upon the scale reached by the fluid, the reading being taken from the *middle* of the concave surface caused by capillary attraction.

VARIATIONS IN THE FORMED ELEMENTS OF THE BLOOD.

RED BLOOD-CORPUSCLES.

Polycythemia.—An increase in the number of red blood-cells occurs in new-born infants, upon a removal from a low country to a high altitude, in those conditions which decrease the amount of blood-plasma,—diarrhea and watery exudates in general,—after the administration of certain drugs, and, as has been mentioned, after exercise and cold baths.

Oligocythemia.—This term is used to denote a decrease in number of the red blood-cells. Such a condition is frequently met with, notably in the anemias, where in the pernicious form they may fall as low as 360,000 to the cubic millimeter, in chlorosis, after hemorrhage, in carcinoma, etc.

Poikilocytosis.—Variations in size and form are indicated by this term. The condition is always present in pernicious anemia and is frequent in secondary anemia from any cause. At times it is scarcely possible to find a corpuscle of normal shape. They are distorted into every conceivable form, but perhaps most often assume a club-like shape with the handle curved and pointed, others are crescentic, many of the cells are much smaller than normal, and all are more or less faded in appearance, owing to the diminished amount of hemoglobin.

The condition once seen is easily again recognized. Care must be exercised not to confound it with the distortion of the corpuscles caused by faulty technique in making the spread. In such a case it will be observed that all of the altered cells are elongated in *one direction*, their

long diameters *lying in the same axis*, which is not the case in poikilocytosis.

Microcythemia.—In anemias, toxic conditions, and after severe burns this condition of the red blood-corpuscles may be observed. It consists in the presence of large numbers of *microcytes*. It has been ascribed by Gräber to the rapid abstraction of fluid from the red blood-cells and a consequent rapid shrinking of these elements.

The condition may appear and disappear with equal suddenness. Clinically it is of little known importance, as its cause and significance are not understood.

Polychromatophilic Degeneration.

Ehrlich has pointed out that in certain conditions many of the red blood-cells have the property after hematoxylin and eosin of staining in an irregular way, there being examples varying from a bluish-red to a violet or even a deep blue. He considers this a form of degeneration. A number of other observers take issue with him on this question, believing that these are young cells in process of development. The subject is still *sub judice*, and therefore of no practical value at present.

Granular Degeneration.—Grawitz first described as a degenerative process the peculiar basophilic granulations that may be noted in the red blood-cells of pernicious anemia, carcinoma, severe malaria, and other diseases. After methylene-blue and eosin they appear as dots or rods of a deep blue color lying within the red blood-cells, while with those dyes that affect chromatin they stain as does this substance. For the latter reason they have been thought to be the remains of nuclear degeneration.

While the nature of the granules is not understood, their presence must be taken as indicating a destructive process of the red cells.

Loss of Staining Reaction.

Upon the amount of hemoglobin present in the red blood-cell its staining power directly depends. As Ehrlich has shown, the general appearance of a stained preparation gives a fair index of the quantity of hemoglobin present, and in the examination of a smear this quality of taking the stain should always be noted.

Deficiency of hemoglobin is shown by the corpuscles being pale and their centers devoid of color. In severe anemias the hemoglobin may be so low that only shadowy outlines of the cells can be seen. In such cases poikilocytosis is usually marked.

Caution: Faulty technique will produce a vacuolation of the cells which must be differentiated from the above. The borders of the vacuoles are sharply defined, and to the practised eye the artifact is patent.

INCREASE IN THE NUMBER OF WHITE BLOOD-CORPUSCLES.

The term leukocytosis was formerly used to designate an increase in number of any or all the forms of white blood-corpuscles. It has been found necessary, as our knowledge of the blood increased, to formulate more specific terms that indicate the kind of white blood-cell the number of which is increased.

The increase may be either relative or actual. We have therefore:

Lymphocytosis:	increase	in	the number of	lymphocytes.
Leukocytosis:	"	"	"	polynuclear leukocytes.
Eosinophilia:	"	"	"	eosinophiles.
Mixed leukocytosis:	"	"	"	several kinds of white blood-corpuscles.

Hypoleukocytosis (leukopenia) indicates a decrease below the normal of the number of white blood-corpuscles.

Hyperleukocytosis is sometimes used to denote increase of white blood-cells, but the term is too cumbersome and is not much employed.

Physiologic Increase in the Number of White Blood-cells.

As has been mentioned, this may occur under a variety of conditions, during digestion, after severe muscular exercise, following a cold bath, for the first few days after birth, during pregnancy, and upon the administration of certain drugs—turpentine, camphor, etc.

Pathologic Increase in the Number of White Blood-cells.

Such an increase is found accompanying a variety of pathologic conditions, and often is of the greatest clinical significance. Aside from the special diseases in which it is a primary factor, the inflammatory type is perhaps the most important.

Leukocytosis (Inflammatory). — Probably the chief source in adult life of the polynuclear cells is the red bone-marrow, but there has been much controversy upon this point, nor is it yet satisfactorily elucidated.

In nearly all of the acute infectious diseases there is a distinct leukocytosis. In this category are included diphtheria, pneumonia, acute articular rheumatism, parotitis, scarlatina, smallpox, and septic conditions from whatever cause.

A notable exception to this exists in the case of uncomplicated enteric fever and measles. In these diseases there is a marked *decrease* of the white blood-corpuscles, chiefly of the polynuclear variety.

Usually the greater the leukocytosis, the more serious is the disease and the less favorable the prognosis.

A leukocytosis accompanying enteric fever is considered an indication of some complication of grave nature.

The increase of the polynuclear neutrophiles may be very considerable. In pneumonia Cabot found an average increase of 24,000 of these elements.

According to Simon, there is in scarlatina a leukocy-

tosis reaching to from 10,000 to 25,000 above the normal upon the second or third day after the appearance of the eruption.

The leukocytosis in carcinoma, and more particularly in sarcoma, has long been noted.

Lymphocytosis.—As the lymphocytes originate in the general lymphatic system, it follows that in conditions affecting the lymphatic glands and channels there will be a deviation from the normal number of lymphocytes in the blood. Such a deviation may be either relative or absolute.

It may be broadly stated that whenever there is a hyperplasia of the lymphatic glands, there will be a corresponding increase in the number of lymphocytes in the peripheral circulation.

Thus, Ewing mentions a case of enteric fever in which there was a lymphocytosis of marked degree and where there were found postmortem greatly enlarged mesenteric glands. So also there is in malignant lymphoma not complicating the lymphatic channels a uniform lymphocytosis.

In case the lymph-streams are blocked, Ehrlich believes the decrease in the number of lymphocytes is due to their inability to gain entrance to the blood-stream.

In whooping-cough lymphocytosis is often marked.

Experimentally it may be induced by the injection of tuberculin and pilocarpin.

Eosinophilia.—The normal number of polynuclear eosinophiles is always greatly increased in myelogenic leukemia, and may occur in many other conditions. Brown and Thayer first pointed out the eosinophilia accompanying trichinosis. In four cases reported by them the eosinophiles present reached not lower than 48 per cent. of the total number of white blood-corpuscles.

The condition may also occur in various skin affections, gout, gonorrhea, and the eruptions of syphilis. In cases of infection by anchylostoma and other intestinal parasites eosinophilia may be extreme, but is not constant.

Leukemia.—This is a general term denoting an enormous increase in the number of white blood-cells dependent upon definite pathologic conditions of the formative centers of these cells.

There is usually a decrease in the number of red blood-cells and in the percentage of hemoglobin. The proportion of white corpuscles to red may in severe cases reach the astonishing figure of 1 : 3.

By some authors it has been claimed that there exist three distinct clinical forms of leukemia: *lymphatic*, with general hyperplasia of the lymph-nodes; *splenic*, with enlargement of the spleen; and *myelogenic*, with corresponding hyperplasia and activity of the red bone-marrow.

Ehrlich contends that it is not possible to distinguish as distinct the first two forms, and classes them together as *lymphatic leukemia*.

Lymphatic Leukemia.

It is now universally admitted that the lymphocytes of the blood are identical wih the fixed lymphoid cells of the lymph-nodes, of whatever variety. It needs, therefore, only a hyperplastic condition of these structures, together with a free access for the cells manufactured, to furnish to the blood-stream an excess of these elements. Such a condition has been noted in cases of malignant lymphoma, —the so-called lymphosarcoma,—in which the number of lymphocytes may be greatly increased. In pertussis during the convulsive stage there may also be a distinct

lymphocytosis, perhaps to four times the normal number of these cells.

In true lymphatic leukemia there are, however, distinguishing features that allow a positive diagnosis to be made with certainty.

Diagnosis.—The blood should conform to the following conditions:

1. A constant excess of lymphocytes and large mononuclear leukocytes amounting to about 150,000 per cubic millimeter and to 80 to 90 per cent. of the white blood-corpuscles.
2. A total absence or the presence in only small numbers of eosinophiles, polynuclear leukocytes, mast-cells, myelocytes, and nucleated red cells.
3. A decrease in the number of red blood-cells, sometimes the number falling as low as 2,500,000 per cubic millimeter.
4. Usually a decrease in hemoglobin.

The internal organs, particularly the spleen, liver, and kidneys, may be greatly enlarged by the infiltration of white blood-cells, which may occur in large aggregations, appearing as white patches upon section, and as nodular masses when lying near the surface.

The author has seen one case of lymphatic leukemia in which the splenic tumor weighed over 4000 grams. The lymphatic structures were uniformly greatly enlarged.

Ehrlich recognizes two types:

Acute lymphatic leukemia, which pursues a rapid course, presents a small splenic tumor, and is characterized by a tendency to the formation of petechiæ and hemorrhage.

Chronic lymphatic leukemia, in which the course of the disease may extend over years, the spleen being often very considerably enlarged.

The diagnosis between these two forms cannot be made

from the microscopic appearance of the blood alone, as in both the proportions and general characteristics of the cells are identical.

Myelogenic Leukemia.

In the range of hematology there is no condition that presents a more typical microscopic picture than the blood of myelogenic leukemia.

The distinctive feature of the disease is the presence in the circulating blood of large numbers of mononuclear neutrophile cells (myelocytes) which normally are found only in the red bone-marrow. There is also a great absolute increase of eosinophiles, both of the polynuclear and of the mononuclear types (eosinophilic myelocytes), and mast-cells are often present in large numbers, while atypical polynuclear neutrophiles are frequent, presenting as dwarfed and distorted forms.

Nucleated red blood-cells are a constant factor and may occur in large numbers. They consist largely of normoblasts, though in some cases megaloblasts are frequent and microblasts may be found without difficulty.

Diagnosis.—The essential characteristics of the blood in myelogenic leukemia are considered by Ehrlich to be as follows:

"1. That, in addition to the polynuclear cells, their earlier stages, the mononuclear neutrophiles, likewise circulate in the blood.

2. That all three types of granulated cells—the neutrophile, eosinophile, and mast-cells—participate in the increase of the white blood-corpuscles.

3. That atypical cell forms—*e. g.*, dwarfed forms of all kinds of white blood-corpuscles—and mitotic figures appear.

4. That the blood always contains nucleated red blood-corpuscles, often in great numbers."

In addition, we may remark that the relative number of myelocytes usually amounts to 20 to 60 per cent., and that the total number of erythrocytes is decreased and there is usually a diminution in the amount of hemoglobin.

CHANGES IN THE BLOOD OF LEUKEMIA PRODUCED BY INTERCURRENT DISEASES.

A reduction in the proportion of leukocytes or myelocytes may be occasioned when there is superimposed some form of infectious disease.

This particularly is true in an intercurrent pneumonia or diphtheria, when temporarily the blood may give the picture of an acute inflammatory leukocytosis.

In early stages of the disease a positive diagnosis may not be possible.

Pseudo-leukemia (Hodgkin's Disease).

In the progressive primary enlargement of the spleen and lymphatic glands known as Hodgkin's disease the blood fails to show the increase in white corpuscles characteristic of leukemia, and this condition cannot therefore be classed as a blood disease.

Any blood changes occurring, *e. g.*, anemia and diminution of hemoglobin, must be considered as purely secondary.

The true lymphosarcomata are so closely allied to the above disease that a clear distinction between them is not at present possible.

A pseudoleukemia of benign type may be transformed into a malignant proliferation of the most pronounced form, there being numerous metastases, invasion of neighboring tissues, and *cachexia*, while microscopic ex-

amination of the tissues shows an undoubted sarcomatous structure.

Pernicious Anemia.

This term is used to designate a progressive pathologic condition characterized by marked changes in the formed elements of the blood—oligocythemia, poikilocytosis—and the presence of megaloblasts and normoblasts and a proportionate increase in the amount of hemoglobin.

There occurs then a progressive *decrease* in the number of blood-cells with a relative *increase* in hemoglobin and nitrogen and also in the size of the red blood-corpuscles. This is believed by Ehrlich to be an effort on the part of nature to compensate for the loss of surface-area of the red cells.

The disease is sometimes due to the presence of intestinal parasites, but more often it apparently is idiopathic in origin and distinct unto itself. Women are more frequently affected with this disease than men.

As exciting causes may be mentioned malaria, tuberculosis, diseases affecting the gastric mucosa, cancer of the stomach, persistent diarrhea, and pregnancy.

Changes in the Blood.—The quantity of blood is greatly decreased, so that it appears thin and watery, and it may be difficult to obtain a drop for examination, a deeper puncture than usual being necessary.

The hemoglobin, while it is relatively increased in the individual blood-corpuscles, is actually reduced, so that in the later stages of the disease it may fall as low as 10 per cent.

Usually the percentage of hemoglobin is between 20 and 40 (Ewing), and it is frequently greater than occurs in secondary anemias from various causes.

The number of red cells may fall very low, in fatal cases

usually about 1,000,000 per cubic millimeter, and cases have been observed in which they reached the low number of about 200,000 per cubic millimeter.

The size of the red cells is greatly increased, so that many of them measure as much as 18 μ in diameter, while the average, as determined by Lazarus, is 11 μ to 13 μ.

According to Ewing, at least 33 per cent. of the red cells should show the increase in size in order to make the diagnosis of pernicious anemia sure.

Megaloblasts constitute the majority of nucleated red cells present. They may occur in large numbers or be found only after careful search.

Microblasts and normoblasts are conspicuous by their absence, being rarely found in the blood in pernicious anemia. Mitotic figures in megaloblasts may be observed, and Ewing asserts that "the presence of three or more unequal asters in one gigantoblast, as occasionally found in severe cases, is one of the most significant signs within the range of blood analysis."

The same author lays down the following rules to be observed in determining the diagnosis of suspected cases:

"*The diagnosis* may rest upon the presence of: numerous megaloblasts and megalocytes with increased Hb; 33 per cent. of megalocytes with increased Hb; an excess of megaloblasts over normoblasts, a single gigantoblast or megaloblast in pathologic mitosis. The diagnosis cannot *rest* on an extreme reduction of red cells. The diagnosis *may require* the complete summation of all clinical and morphologic data, as well as observation on the course of the disease, or even the microscopic examination of the marrow."

Secondary Anemia.—Diminution of the number of red blood-cells and of the hemoglobin is of frequent occurrence

after severe hemorrhage and following tuberculosis, chronic nephritis, and various infectious diseases.

The changes described as occurring in pernicious anemia are absent; that is, the blood does not contain megaloblasts, the hemoglobin is not relatively increased, and the red bone-marrow fails to show the hyperplastic changes always present in pernicious anemia.

Chlorosis.

This term is applied to a primary anemia peculiar to young females, rarely existing in the male. It is characterized by a deficiency of hemoglobin without a corresponding diminution of red blood-cells.

From progressive anemia it differs in the above respects, and also in that while nucleated red cells may occur in small numbers the presence of megaloblasts in greater proportion than normoblasts is absent; in fact, the presence of megaloblasts in the blood of chlorosis is denied by some authors. Blood-plates are much increased in number and the coagulability of the blood is greater than normal.

The female sex and the age of puberty seem to be important factors, while heredity and tuberculosis also play a part in the etiology of the disease.

Microscopic Appearance of the Blood.—The red blood-cells are slightly decreased in number, though rarely the count may fall to about 2,000,000 per cubic millimeter.

Poikilocytosis and oval forms are frequent in the severer forms and the cells are notably pale, the centers frequently being quite clear on account of deficiency in hemoglobin.

In cases of moderate severity the percentage of hemoglobin is usually about 40, while in severe cases it may fall much below this mark.

Leukocytosis.—Usually the number of white blood-cells remains unchanged.

Malaria.

The specific cause of malaria was discovered in 1880 by Laveran, who observed in the blood of malarial patients a crescentic parasite, which was in his honor called *Laverania malariæ.*

The malarial organism belongs to the *Protozoa*, the lowest class of animal forms. It is endowed with the power of ameboid movement and possesses a nucleus. Multiplication takes place by division of the adult forms.

The question whether there are several distinct varieties of malarial parasite, or only one species differing widely in its morphology and producing diverse phenomena under varying conditions, is one that has claimed a great deal of attention. It must still be considered unsettled. By most of the German and American authorities the theory of plurality of species is considered established. The theory that there is but one form of malarial organism is strongly maintained by Laveran and many of the Italian observers.

The author prefers to accept the classification of the malarial parasite into three distinct forms—the tertian Hæmamœba vivax, the quartan Hæmamœba malariæ, and the estivo-autumnal Hæmamœba falcipavum.

Morphology and Biology.—Appearance in Unstained Preparations: After segmentation of the adult parasite the subdivisions or *spores* first adhere to the red blood-corpuscles and soon after gain entrance to them, the subsequent development being *endoglobular.*

In the first stage the young malarial parasite is seen in fresh, unstained blood as a small, spheroidal, hyaline body within the red blood-cell, in which it is usually excentrically placed. It measures about 1 μ in diameter, and therefore occupies about one-tenth part of the erythrocyte.

It is at first not sharply differentiated from the sur-

rounding greenish-yellow protoplasm of the blood-cell, but merges with it so gradually that no distinct line of demarcation can be seen. Its position in the corpuscles may change, but the activity displayed by the older forms is usually not present.

Within a few hours after the chill a notable increase in the size of the organism has taken place, and usually it has become ring-shaped, there being a central portion which shows the color characteristic of the normal protoplasm of the red blood-corpuscle.

Active motility may now be observed provided the specimen is freshly prepared and the temperature of the room is not too low or a warm stage is employed. The organism is seen to rapidly change its shape, sending out finger-like projections of its substance (*pseudopodia*), which may be as rapidly withdrawn, to be again shot forth in another direction.

The whole organism may change its position in the red blood-cell, this being accomplished by the advancement of a pseudopod and the subsequent withdrawal of the remainder of the organism into the projected portion.

The organism may frequently be seen to change its relation to the surface of the blood-cell, the pseudopodia dipping down to a lower plane, thus apparently disproving the assertion of some observers that at this stage the organism is simply attached to the corpuscle and not within its substance.

As the parasite increases in size fine pigment-granules appear, their number increasing with the age of the organism. The pigment is of a dark brown color and frequently occurs in rod-shaped masses and in cubes, varying with the age and kind of organism.

Great activity of the pigment is usually present. The particles exhibit a rapid dancing motion, forcibly re-

minding one of Brownian movement, with which, however, it is not identical, being due to the protoplasmic activity of the organism.

Presegmenting Forms.—As the malarial organism approaches maturity it occupies the greater part of the red blood-corpuscle and assumes a spherical shape, the pigment becoming at the same time less motile, and finally arranging itself more or less peripherally and ceasing its movements entirely.

These *presegmenting* forms occur from six to eight hours before the chill, and a few may even be found within an hour or two after the chill, these representing organisms that have not kept pace with the uniform development of the majority.

Segmentation.—Two or three hours prior to the chill segmenting forms begin to appear, becoming more and more numerous as the time of the paroxysm approaches, and being most abundant just before it begins. As is the case with the presegmenting forms, the segmenting bodies may be found in small numbers sometimes as long as several hours after the chill has taken place.

These adult forms consist of a number of perfect spores arranged in the form of a rosette, so that the outline of the organism is regularly lobulated, there being around the whole structure a thin zone of pale yellow color, the remains of the red blood-corpuscles.

The organism itself is highly refractile and exhibits a faint rosy tint; the line of demarcation between the component spores may be quite indistinct except at the edges or there may be a radial striation that is quite easily made out. Each of the spores is seen to contain a small dot marking the site of the nucleus. The pigment is usually arranged centrally in an irregular mass.

The organism now bursts from the inclosing blood-

corpuscle, the individual spores are set free and probably soon attach themselves to the red blood-corpuscles to begin afresh the cycle of existence while the pigment either remains free in the blood-plasma or more probably is taken up by the leukocytes.

Multiple infection of a single red blood-cell is frequently observed in all forms of malaria, but more particularly in the estivo-autumnal type.

Flagellated Bodies.—Extracorpuscular bodies with long projections of their protoplasm (flagella) may occasionally be noted, and may be produced artificially by the methods to be mentioned (see Technique). They probably represent degenerative forms and are incapable of reproduction.

The flagella are often so active that they can scarcely be seen. Within the radius of their activity the blood-plasma is set in violent motion and pigment-granules or red blood-corpuscles drifting within their reach are dashed aside by the whip-like motion of the delicate processes. As the organism becomes less active the flagella are seen to be several times longer than the diameter of the body, and frequently they possess a club-like extremity.

Crescents and ovoids are found only in estivo-autumnal infection, and will be considered under that head. Their detection in fresh blood is a matter of ease on account of their peculiar shape and the presence of a considerable amount of pigment, usually centrally located and arranged in masses.

Organisms in the Peripheral Blood.—The malarial parasite may be present in the internal organs and bone-marrow in enormous numbers, and at the same time so few may appear in the peripheral circulation as to be difficult of demonstration.

This fact should not be lost sight of. Care and perse-

verance will enable the competent observer to find the malarial parasite in blood from the peripheral circulation in any case of malaria with an infection of sufficient intensity to give rise to clinical manifestations.

Leukocytes in Malaria.—The phagocytic action of the white blood-cells is in malaria of particular interest and value. The fact that leukocytes are present, containing the characteristic pigment-granules and bars or blocks formed by the malarial organism during its development, is, of itself, sufficient data upon which to base a positive diagnosis. This fact is of great aid to the clinician in cases where the organisms are not present in quantity in the peripheral circulation or where the administration of quinin has reduced or obliterated the infection.

In fresh preparations the process of phagocytosis may often be actually seen. Sometimes scarcely a leukocyte can be found that does not contain a recently engulfed malarial parasite which may still be alive with its pigment-granules in active motion.

The author has several times observed the process, which is interesting in the extreme. The leukocyte by ameboid movement slowly approaches the malarial parasite until it is, as it were, within striking distance of it. A pseudopod is quite rapidly thrown out until it touches the parasite, the pigment-granules of which seem to be immediately set in more active motion, as if the parasite realized its danger. The leukocyte, on the other hand, quickly withdraws the pseudopod and retreats a short distance. It gives the impression of having received a rebuff and of fortifying itself anew for the attack.

Again it advances and projects a pseudopod, which is again withdrawn, but with less rapidity than in the first instance, nor does the leukocyte remove itself so far from its prey as in the first retreat.

Each time that it advances it seems to do so with more assurance, and the pseudopod remains longer in contact with the malarial organism, until finally it is no longer withdrawn and the white blood-cell literally crawls around the parasite. After having engulfed its prey the leukocyte may exhibit sufficient energy to travel out of the field of vision unless the slide be moved.

This phagocytic action has been observed in several cases of estivo-autumnal type exhibiting large numbers of advanced extracorpuscular forms. From the action of the leukocyte it would seem that the malarial organism possesses some power of resistance which must be gradually overcome by the leukocyte before inclosure can take place.

Technique.—Before essaying the study of the malarial parasite one must thoroughly familiarize himself with the appearances of normal blood in both the fresh unstained and stained specimen, so that he will have become acquainted with those artifacts that continually arise with even the most careful technique.

It goes without saying that he must have a thorough knowledge of the normal elements of the blood and their behavior toward the various staining methods employed. Continued and careful observation is the only qualification possible.

Wet Preparations.—Though the technique is simple in execution, the beginner is apt to fail to make proper preparations of fresh blood. A little experience will soon remedy his defects. Careful attention to details is essential.

The lobe of the patient's ear is cleansed with soap and water and wiped dry with a clean towel or piece of gauze. A puncture is made with the point of a small sharp-pointed tenatome or other suitable instrument, so that

with slight pressure a drop of blood will flow. If the lobe is held between the thumb and finger and submitted to considerable pressure, the slight prick necessary will scarcely be felt by the patient.

The first drop is wiped away, a cover-glass held in the forceps is touched to the succeeding drop and is quickly placed "butter-side down" on a glass slip. The blood should spread quickly and evenly between the two surfaces. This action may be facilitated if necessary by *gently* tapping the edge of the cover-glass with the point of the forceps. Pressure upon the cover-glass must never be made.

Both the cover-glass and slip must be previously scrupulously cleaned. This is best accomplished by immersing them in alcohol and wiping them with a clean piece of gauze. Immediately before the preparation is to be made they should be vigorously rubbed with a clean cloth. If the slip is submitted to this friction until it is warmed, the blood spreads the better. The slightest piece of dust upon the cover or slip is fatal to a good result. The smallest amount of acid will ruin the preparation. Glass that has been cleaned in an acid bath must therefore be carefully washed with soap and water.

After the slips and covers have been cleaned by ordinary methods, it is not amiss to pass them several times through the bunsen flame in order to remove any grease that may remain.

Examination of Fresh Blood Preparations.—The fresh spread should be examined as soon as possible, as changes in the blood may soon occur. If the preparation is not to be viewed immediately, it is well to seal the edge of the cover with oil or vaselin to prevent evaporation.

A drop of immersion oil having been put upon the cover-glass, examination with the $\frac{1}{12}$ objective is pro-

ceeded with. The specimen should, if properly made, exhibit, at least in certain portions, a thin stratum of blood-cells lying flat and separated slightly one from the other. If the cells have clumped or have formed rouleaux, it signifies that too large a drop of blood has been used. Usually the spread is thickest at the edges or upon one side.

It is sometimes advisable to search these thicker portions of the preparations, for we may there find the larger pigmented organisms after hunting in vain for them elsewhere.

The fresh preparation is of advantage when we wish to study:

1. Ameboid and pigmentary movements.
2. Flagellated bodies.
3. Phagocytosis.

Flagellated Forms.—In order to observe these bodies it is necessary to liberate the adult parasite from the restraining bounds of the red blood-cell. The escape of the organism may be facilitated by adding to the fresh preparation a small drop of water. This should be placed upon the slide at the edge of the cover-glass, under which it will be drawn by capillary attraction.

Ewing recommends that fresh smears be placed immediately in a moist chamber (and the latter quickly sealed) for from ten to twenty minutes.

Dried Blood Preparations.—The technique for making and fixing smears is fully described in the section on blood (p. 242).

Stains.—Eosin and methylene-blue form one of the most satisfactory methods for diagnostic purposes. Two solutions are required: (1) Saturated alcoholic eosin; (2) saturated aqueous methylene-blue.

The smear, having been fixed in methyl alcohol or

formalin and alcohol, is stained with the eosin solution for ten seconds, washed in water and stained for thirty seconds with the methylene-blue, washed, blotted, dried, and mounted in Canada balsam or examined without a cover-glass, a drop of immersion oil being placed directly upon the stained blood. By this method the malarial organism and all nuclei of white blood-cells are stained blue, the erythrocytes being a bright red.

Carbol-thionin.—This is a most serviceable stain, for the reason that the ring forms are sharply brought out by it, while with the methylene-blue they may stain faintly.

The stain is made as follows, after the formula of Futcher and Lazear:

Saturated solution of thionin in 50 per cent. alcohol,..... 20 c.c.
2 per cent. aqueous carbolic acid solution, ..100 c.c.

The mixture must be at least one week old, and improves with age. After fixation with alcohol and formalin or methyl alcohol, stain thirty to sixty seconds. Wash, dry, and examine either direct or after mounting in Canada balsam.

The above stains suffice for the easy identification of the organism, but neither of them furnishes a differential stain of the chromatin of the nucleus. In order to accomplish this purpose, which is desirable for critical study of the malarial parasite, recourse must be had to some one of the stains in which polychrome methylene-blue is employed. Goldhorn has recently formulated an excellent stain for this purpose. It may be purchased from any of the larger dealers in microscopic supplies.

The method of application is as follows:

The smears must be freshly made.

Fix in methyl alcohol fifteen seconds.

Wash in water.

Stain seven to thirty seconds in $\frac{1}{10}$ of 1 per cent. aqueous eosin solution.

Wash in water.

Stain thirty to sixty seconds in Goldhorn's methylene-blue.

Wash in water.

Dry, without the use of blotting-paper or heat, by agitation of the slide in the air.

Besides staining the chromatin reddish, the body of the parasite is stained blue, nuclei of leukocytes blue, and the granular degeneration of infected red blood-corpuscles is beautifully shown. Advantages of stained specimens are:

1. Easy detection of existing parasites.

2. Structural details of parasite are brought out (with Goldhorn stain).

For routine work stained specimens are far superior to the fresh preparation. They are particularly recommended to the beginner in malarial parasitology.

Tertian Parasite.—The life-cycle of this form of the malarial parasite is about forty-eight hours.

It develops in well-marked groups, the individuals of which mature about the same time. An infection with such a group of tertian parasites results in a rise of temperature with a chill at the period of sporulation, so that we find the paroxysm appearing upon every third day. This is followed by a rapid fall of temperature, which usually becomes subnormal, the parasites at this stage being in the young, hyaline form. There follows a period of quiescence, the temperature remaining about normal the second day.

The organisms will now be found in the ring form with beginning pigmentation. Upon the succeeding day they

again reach maturity and produce a sudden exacerbation of temperature.

Not infrequently there may be present two groups of tertian parasites (double infection). For some unknown reason when this is the case the groups usually mature about twenty-four hours apart. It will readily be seen that this would bring about a paroxysm every day—a quotidian fever. Microscopic examination of the blood about the time of chill would reveal one set of organisms half-grown, the other full-grown, with presegmenting forms and rosettes.

It may even happen that more than two groups of tertian organisms are present in the blood of the same individual, in which case an irregular temperature curve would result.

The tertian parasite in stained preparations shows the following characteristics; it is presumed that a method (as Goldhorn's) that stains the chromatin has been used:

The youngest forms are identical with the recently divided portions of the segmenting bodies. They are nearly spherical, about 1 μ in diameter, the outer portion stains with methylene-blue and incloses a globular red nucleus which is surrounded by an achromatic belt—"the milky zone" of Gautier.

Within a few hours after the paroxysm these forms have been replaced by more or less characteristic rings which may reach 4 μ in diameter. These may already contain a few fine pigment-granules of a reddish-brown color. The center of the ring is occupied by the substance of its corpuscular host.

"The chromatin is usually found within the ring, sometimes lying in an isolated position in the center, but very often the chromatin is found *outside* the ring, connected by a very fine thread of protoplasm" (Ewing).

The parasite continues to increase in size, pigment-granules multiply, the chromatin is subdivided into small granules, ten or more in number, which lie in a group surrounded by a "milky zone." The organism at this stage is very variable in form, and may stretch out long threads in various directions, at times spanning the entire red blood-cell.

About ten hours before the chill the parasite seems to have reached its full size. It stains quite homogeneously, pigment-granules and bars are present in large numbers throughout the body of the organism, and the larger "milky zone" is occupied by many small granular masses of chromatin.

Presegmenting bodies now begin to be seen. With methylene-blue and eosin these forms are readily distinguishable on account of their capacity for taking the stain deeply and presenting a reticulated appearance. A chromatin stain discloses numerous coarse granules of chromatin scattered in groups throughout the body of the parasite, each group being surrounded by a "milky zone."

Segmenting Forms.—Gradual fusion of the chromatin granules takes place until there are formed fifteen to twenty (Thayer) chromatin masses, each surrounded by a "milky zone" representing the nucleus of a new segment. This pigment collects in a mass, usually in the center of the organism, the peculiar striated appearance of the rosette is clearly visible, and the lobulated rim of the organism is surrounded by a thin border of the degenerated red corpuscle.

Changes in the Infected Red Blood-cell.—In the tertian form of infection the erythrocyte attacked by the spore soon shows granular degeneration. As the parasite grows, the red blood-corpuscle increases in size and becomes progressively paler. This is due partly to the de-

generative process and partly to the fact that the hemoglobin, upon which its staining qualities depend, is being rapidly converted into melanin. Toward the latter part of the cycle of the parasite the red blood-cell may have increased to nearly twice its normal size.

The Quartan Parasite.—The cycle of development of the quartan organism lasts about seventy-two hours. In an infection by a single group of this form of the parasite there would therefore be a rise of temperature with a chill upon every fourth day.

An infection by two groups of quartan organisms would produce a paroxysm upon two succeeding days, with an intermission of one day, while an infection by three groups of the quartan parasite maturing upon successive days would result in a quotidian type of fever.

The quartan form of malaria is comparatively rare in America, so far as is known from present observation.

Morphology of the Quartan Parasite.—Stained specimens of the younger forms are apparently identical with those of the tertian type.

After a few hours it is not difficult to distinguish the one from the other. The quartan organism remains small, more rotund, and much more refractive in fresh preparations than the tertian. The pigment-granules are fewer in number, larger, and darker, the movement of the parasite is at all times slower than that of the tertian, and the pigment is sluggish or nearly stationary.

Presegmenting Forms.—These are frequently characterized by a radial or star-shaped arrangement of the pigment, and the reticulation is coarser.

Segmenting Forms.—On account of the slower development of the quartan parasite rosettes are more frequent in the peripheral circulation than in either of the other forms of malaria.

They present only from 6 to 12 segments, and are consequently much smaller than those of the other varieties.

The infected red blood-cell in quartan malaria is quite different in its appearance from that of tertian. Instead of increasing in size and becoming paler, it is diminished, and in fresh preparations of a peculiar brassy hue. Nor are its staining qualities so notably affected.

The Estivo-autumnal Parasite.—Certain irregular forms of malarial fever, occurring chiefly in the late summer or autumn, present a form of parasite differing essentially in many particulars from the ones already described. The paroxysms may occur with regularity about forty-eight hours apart, but frequently vary considerably, so that we may have a typical quotidian range of temperature with chill every twenty-four hours.

Paroxysms may, indeed, occur at intervals ranging all the way between the two extremes. In the more serious types of this form of malaria the paroxysms are usually tertian, while the milder forms are prone to follow with greater or less regularity the shorter interval. There is also a notable difference in the time the paroxysms of estivo-autumnal fever persist; they may last as long as thirty-six hours, and are commonly of twenty-four hours' duration, while in the fever due to the tertian and quartan organisms the paroxysms are of much shorter duration, rarely exceeding ten or twelve hours. Again, in the remittent types the paroxysms may merge one with the other, so that the temperature does not at any time reach the normal point.

It is a peculiar fact that while the parasites may exist in myriads in the internal organs, notably the spleen and bone-marrow, they may not be demonstrable in the peripheral circulation after the first few hours of their existence. Neither do they form well-defined groups of

organisms of about the same age and stage of development. Besides, they present morphologic characteristics peculiar to themselves.

On account of the variations in the clinical manifestations of infections by the estivo-autumnal parasite, and because of real or fancied deviations in form, Marchiafava, Bignami, Celli, and others have contended that there are at least two distinct species of this variety, the one producing the milder tertian, the other the more serious quotidian and continued fevers.

While there may be reasonable ground for this belief, the majority of observers are inclined to the opinion that there is but one form, which is capable of producing those diverse manifestations on account of varied environment and other reasons at present not understood. To this opinion we will adhere, without entering into a discussion of the merits of either side of the case.

Morphology and Biology.—Except that they are somewhat smaller, the earliest forms of the estivo-autumnal parasites are not distinguishable from those of the tertian and quartan forms.

At an early stage they form rings possessing a marked thickening upon one side, and are therefore distinguished as "signet-ring" forms; these stain particularly well with carbol-thionin. According to Ewing, they nearly always present two nuclear bodies staining with methylene-blue and lying either close together or opposite to each other.

The ring forms gradually increase in size until they are 3 μ to 4 μ in diameter at the end of about twenty-four hours, the thicker portion of the ring increases in size, and fine pigment-granules begin to appear. Presegmenting bodies now begin to be seen which resemble those of the

tertian organism, except that they are of smaller size, often being not more than half the diameter of a normal red blood-corpuscle.

Segmenting Forms.—These are similar to those of the tertian parasite, but according to most observers differ from them in the number of spores formed, there being from 6 to 12, though in cases observed by Ewing nearly all of the rosettes showed from 18 to 20 spores.

Crescents and Ovoids.—Besides the forms above described in estivo-autumnal infections there appear after a few days bodies of ovoid or crescentic shape. They occupy red blood-cells or have clinging to them the remains of the original host and contain pigment bars which may be centrally grouped in an irregular star-shaped figure and motionless in fresh preparations, or may present considerable activity and be scattered throughout the organism.

The author has upon several occasions observed the active pigment of a crescent withdraw itself from the extremities of the body and assume the characteristic central arrangement. That the ovoids may become converted into crescents, and the latter under proper conditions frequently revert to the oval form, is well established. The genesis of the bodies is still a matter of doubt, despite much study and contention regarding it.

Chromatin granules in the crescents have been demonstrated by a number of observers. They usually appear in the center of the body, among the pigment staves.

Ovoids and crescents persist in the peripheral blood for weeks after the other forms have disappeared and the patient has apparently recovered. In one such case observed by the author there was a relapse after six weeks, following apparent recovery, the patient succumbing to the second attack.

The question as to whether or not the ovoids and crescents influence the febrile movements or partake in the process of perpetuation of species is as yet not satisfactorily answered. The fact that these forms contain numerous chromatin granules would incline one to the belief that they are not devoid of the reproductive faculty under favorable conditions.

Each of these forms may become extracorpuscular and flagellated.

Extracorpuscular Bodies.—In fresh preparations that have been allowed to stand for several hours large, swollen, hyaline bodies of spherical form may frequently be observed. They possess pigment-granules which display considerable activity, but with the proper stains show little or no chromatin. They are probably the results of changed environment, and do not properly belong to the normal cycle of development of the parasite.

The Red Blood-corpuscle.—The changes occurring in the erythrocyte infected by the estivo-autumnal organism are even more marked than those occurring in tertian and quartan infections. The red cell increases in size at an early stage, and may become wrinkled and crenated, while in fresh blood it assumes a greenish-yellow hue. Granular degeneration and diminished staining reaction are prominent and early manifestations. Multiple infection of a single red cell is more frequent in the estivo-autumnal than in other varieties.

The red cells in which a crescent has developed rapidly lose their hemoglobin in the vicinity of the organism, until finally only the outer border of the "bib" will respond to the stain. This results in the appearance, so frequently seen in connection with these bodies, of a faint, red line, either smooth or irregular in outline, connecting the horns of the crescent and bellying out from its concave

sides. At times a similar line borders the convex edge, which it usually hugs closely.

Diseases frequently confounded with malaria are enteric fever, miliary tuberculosis, and septic infection. The microscope usually affords a ready means of diagnosis between these maladies.

TABULATION OF DISTINGUISHING FEATURES OF VARIETIES OF MALARIAL PARASITES.

TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL.
1 Life-cycle about forty-eight hours.	1. Life-cycle about seventy-two hours.	1. Life-cycle twenty-four to seventy-two hours (uncertain).
2. Ring forms, nucleus *not* stained by methylene-blue.	2. Ring forms, nucleus *not* stained by methylene-blue.	2. Ring forms, nucleus *stained* by methylene-blue.
3. Ring forms irregular and thick.	3. Ring forms similar to tertian but smaller.	3. Ring forms more delicate and "signet" portion more marked.
4. Presegmenting forms large and fill the red blood-cell.	4. Presegmenting forms smaller than tertian.	4. Presegmenting forms smaller than tertian.
5. Rosettes 15 to 20 or more spores.	5. Rosettes 6 to 12 spores.	5. Rosettes 6 to 12 spores.
6. Pigment-granules fine, numerous and active. Central arrangement in rosettes.	6. Pigment coarse and sluggish. Stellate arrangement in rosettes.	6. Pigment-granules usually coarse and sluggish. Central arrangement in rosettes.
7. Infected red blood-cell grows larger and becomes pale.	7. Infected red blood-cell becomes smaller and brassy.	7. Infected red blood-cell becomes smaller, brassy, and rapidly loses its hemoglobin.
8. All forms in peripheral circulation.	8. All forms in peripheral circulation.	8. Late forms frequently absent from peripheral circulation.
9. Common, disease mild in character.	9. Rare, disease mild in character.	9. Common in tropics and southern United States. Disease frequently fatal.
10. Rapidly disappear after quinin.	10. Rapidly disappear after quinin.	10. Quinin not always efficient.
		11. Ovoids and crescents occur.

BACTERIA IN THE BLOOD.

In cases of septicemia and pyemia various micro-organisms may be found in the blood-stream, often in considerable numbers. It is frequently not possible, or at best difficult, to demonstrate the bacteria by microscopic examination alone. We must therefore resort to cultural methods.

Agar slants or plates are to be inoculated with about 1 c.c. each of blood taken from a vein of the patient's arm with a hypodermic syringe. The field of operation must be rendered *surgically clean* in the usual manner and the needle sterilized by boiling. The vein may be rendered conspicuous by a ligature or by compression with the finger at a point above that chosen for insertion of the needle.

The operation is simple and painless, and no evil results follow if cleanliness is observed. The inoculated medium is incubated and further procedures carried on as indicated in the section on bacteriology. In glanders and anthrax infections it is usually easy to demonstrate the bacteria by direct microscopic examination.

Smears made on the slide (see p. 242) are prepared as follows:

1. Fix in methyl alcohol thirty seconds.
2. Saturated alcoholic eosin ten seconds.
3. Wash.
4. Methylene-blue (saturated aqueous solution) thirty seconds.
5. Wash.
6. Dry and examine with a $\frac{1}{12}$ objective.

Lipemia.—Fat is found in the blood in cases of fracture of the long bones with laceration of the tissues (embolic

lipemia), and occasionally in severe cases of diabetes, chronic alcoholism, nephritis, malaria, and other diseases.

Method.—Make spreads upon the glass slip, which has been cleaned with ether and alcohol, and when they have dried add a drop of 1 per cent. osmic acid and a cover-glass. Examine with the $\frac{1}{6}$ objective. If fat is present, the droplets will be stained black by the osmic acid.

The lobe of the ear should be cleaned with alcohol and ether before making the puncture in order to cleanse the surface of any fat that may be present.

Ether added to a blood-slide containing particles of fat will dissolve them. This may be used as a confirmatory method.

Micro-chemical Test for Blood.—Dried blood treated in the following manner will produce small, dark brown, rhomboidal, discrete crystals of melanin, which may also

FIG. 105.—HEMIN CRYSTALS.

occur in irregular masses, the free ends of the projecting crystals being cut obliquely.

Method.—

1. A minute quantity of dried blood and a *small* crystal of sodium chlorid are placed on a glass slide.

2. The two are powdered and mixed.
3. A cover-glass is added.
4. A drop of glacial acetic acid is allowed to run underneath the cover-glass.
5. The slide is held over a low bunsen flame until the glacial acetic acid begins to bubble.
6. Set aside to cool.
7. Examine with the $\frac{1}{6}$ dry objective.

Under certain rare conditions the crystals may fail to form. This method may be exceedingly valuable in medicolegal cases.

Filaria sanguinis hominis.

This is the larval stage of a filiform worm which inhabits the lymph-vessels, Filaria Bancrofti. There are a

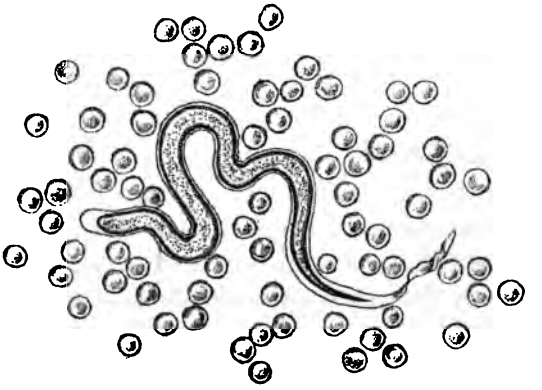

FIG. 106.—FILARIA SANGUINIS HOMINIS.

variety of species which are similar in their habits and produce the same lesions. Similar organisms occur in the blood of birds. The filaria is much more plentiful in the peripheral blood at night, as was first pointed out by Manson.

"It is 0.007 mm. to 0.0011 mm. in breadth and 0.27 to 0.34 mm. long. It has a short, rounded head with a tongue-like appendage, and a long pointed tail. From the hinder extremity a ribbon-like mass projects, and when viewed with the high power of the microscope this, like the cephalic appendage, is seen to be at the end of a closed sac in which the animal can coil or extend itself. This envelope is entirely structureless, but the contained parasite is seen under a very high power to be transversely striated and very granular" (von Jaksch).

The filiara is common in some tropical countries and is not infrequently seen in the blood of patients dwelling along the lower Atlantic coast of the United States.

In the blood it produces no changes, and even when present in large numbers may be unsuspected. The lesions caused by it are mechanical in origin, being due to blocking of lymph-vessels or perforation of blood-capillaries, followed by inflammatory changes, chyluria, or hematuria.

Distoma hæmatobium.

According to von Jaksch, this parasite is found only in the greater cavities of the body, and is therefore rarely seen microscopically during the life of the patient. It is common along the north and east coasts of Africa and has been observed in South America.

The female is 16 to 20 mm. and the male 12 to 14 mm. in length, the latter being thicker than the former and each possessing oval abdominal suckers anteriorly. The eggs are 0.12 mm. long and 0.04 mm. in width and exhibit a spike-like projection from one side. They cause ulceration of mucous surfaces and may give rise to severe diarrhea or hematuria.

URINE.

The urine is the excretion of the kidneys. The normal quantity voided in twenty-four hours may vary within wide limits, dependent upon the amount of liquids consumed, amount of skin excretion, and other well-known factors. For the healthy male it may be said to be about 1500 c.c., while in the female it is somewhat less. Normal urine which has just been passed is a clear, or faintly cloudy, pale yellow fluid having an acid reaction and a specific gravity of 1.015 to 1.025, according to the total quantity passed in twenty-four hours.

Naturally, if the quantity of water is decreased the percentage of solids is relatively higher and there is a corresponding increase in the specific gravity. If the quantity of water be increased above the normal, or if there is a decrease of the solid factors of the urine without a corresponding diminution of the fluid portion, the specific gravity will be lowered.

On account of the fact that the constituents of the urine are not relatively constant at all hours of the day or night, one should always demand that a specimen submitted for examination be taken from a mixed collection of the total urine for twenty-four hours.

The amount of solids in each 1000 c.c. of urine can be roughly estimated by multiplying the last two figures of the specific gravity by 2.33. The result equals the number of grams of solids in 1000 c.c. of urine. The average normal specific gravity being 1020, it follows that 20×2.33 equals 46.60 grams of solids to each 1000 c.c. of urine. If, then, we wish to determine the total amount of solids passed in twenty-four hours, knowing the total

quantity of urine in a given case to be 1500 c.c., the equation would be:

$$\frac{46.60 \times 1500}{1000} = 69.9 \text{ grams of solids.}$$

If the first examination of urine from a suspected case of renal disease is productive of negative results only, this is not sufficient ground upon which to base a positive opinion, as in some forms of Bright's disease the urine may for days be devoid of both albumin and casts. A second or even a third specimen should in doubtful cases always be required.

MICROSCOPIC EXAMINATION OF THE URINE.

Every urine on standing shows a precipitate of varying character and amount. The following table, after Simon, shows the various chemical precipitations that take place in normal urine, dependent upon the reaction of the urine:

Reaction Acid:
- Uric acid.
- Urate of sodium.
- Oxalate of calcium.
- Primary calcium phosphate.
- Ammonio-magnesium phosphate.

Reaction Alkaline (referable to fixed alkalies):
- Secondary calcium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.
- Ammonio-magnesium phosphate.

Reaction Alkaline (referable to ammonia):
- Ammonium urate.
- Ammonio-magnesium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.

Besides these substances, urine in pathologic conditions may contain other substances to be described.

Sedimentation.—This may be accomplished by allowing the urine to stand, either in a clean bottle or in a conical glass. The addition of a small amount of chloroform will prevent the formation of the putrefactive bacteria and the consequent destruction of organic elements present. After the lapse of twelve to twenty-four hours the sediment that will have gathered is first to be inspected *macroscopically*, and some simple chemical tests may reveal the nature of the deposit present, though *microscopic* investigation should always follow. If it is light in color and flocculent, it probably consists of mucin containing a few white blood-cells and epithelium from the lower genito-urinary tract.

Uric acid will give a granular, reddish-brown sediment, sometimes as large as grains of sand. The particles will form upon the sides as well as the bottom of the container.

Urates in an amorphous form frequently occur as a heavy, pink deposit. The urine will be acid, and upon heating in a test-tube the precipitate will rapidly disappear, returning when the specimen is cooled.

Earthy phosphates, carbonates, and alkaline urates may form in alkaline urine as a heavy, white, flocculent deposit, or be precipitated by heat. If the specimen is acidified with nitric or acetic acid, they rapidly disappear.

Pus forms a white, viscid deposit, that resists the action of heat and acids and is rendered ropy or mucilaginous upon agitation with a piece of caustic soda.

Blood in quantity renders the urine pink or reddish-brown and is easily detected with the microscope.

Owing to the fact that the unorganized sediments are usually of little clinical importance, the centrifuge is a useful instrument, as by its aid we can gain the organized elements from fresh urine without loss of time and before they become affected by micro-organisms, and are also

enabled to determine whether bacteria exist in some portion of the urinary tract, normal urine in the bladder being sterile.

The manipulation of the instrument is simple, and as it is of service in quantitative chemical urinalysis, no physician can afford to be without it. A number of forms are made and sold by various dealers. That in which the motive power is water is convenient where water-pressure is accessible, but the ordinary hand apparatus answers every purpose.

How to Work.—For the examination of urine it is convenient to use a large glass slip (2 × 3) upon the center of which a quantity of sediment is placed by the aid of a clean pipette, and spread in a thin layer.

The low power ($\frac{2}{3}$ obj.) is first used, the microscope being in the vertical position and the clips removed in order to give greater range of motion to the slip. *No cover-glass is necessary.* After examination with the low power the $\frac{1}{6}$ may be used if necessary, but to the experienced observer this is generally not the case. Should the front of the objective come in contact with the fluid, it should be immediately removed, wiped dry, and refocused, the layer of urine being spread thinner if necessary.

No attention is to be paid to the formation of crystalline substances where the urine has dried upon the slide. They are seldom characteristic and are heterogeneous in character.

Light.—Of great importance is the proper quality and quantity of light employed. This can only be learned by experience. As a rule, one should, by the manipulation of the mirror and diaphragm, cut down the amount of light to a minimum consistent with a clear picture of the field. Urinary sediments both organic and inorganic are small and more or less transparent, and a brilliant light renders

them invisible. Especially when searching for tube-casts is caution in this respect necessary, as hyaline casts are entirely obliterated by any but a very subdued light.

Chemical Sediments.—In normal urine these substances are usually held in solution. An overproduction of the chemical body or a decrease in volume of solvent powers of the urine will result in their deposit in amorphous or crystalline form. They may be in solution as the urine is passed and deposit as it cools. Sedimentation, either by the centrifugal machine or by allowing the urine to stand in a conical vessel, is usually necessary unless the amount of amorphous or crystalline matter is very large.

Uric Acid.—This deposit occurs in sharply acid urine as a brick-dust sediment which microscopically is seen to consist of crystals of various shapes, chief among them being the rhombic prism, the clusters and rosettes which so often occur being modifications of this form. They are beautifully colored, being deep yellow or orange red, and are totally unlike any other crystalline bodies found in the urine. There is a tendency on the part of these crystals to form upon the sides of the vessel and upon any foreign body that may be present, so that long strings of them may be found attached to a thread or hair. As pointed out by Purdy, this faculty renders the formation of uric acid calculi all the more likely. *Whetstone* crystals and dumb-bell forms are not infrequently observed.

The presence of uric acid crystals does not by any means signify an increase in the elimination of this substance. As has been pointed out, it may be dependent upon a decrease in the watery elements of the urine or the cooling of the urine beyond the point where all the uric acid present can be held in solution. Quantitative chemical analysis only can determine the exact proportion present in the case of this and other chemical sediments. All urine de-

posits its uric acid if allowed to stand and become ammoniacal.

Chemical Significance.—The chief condition of importance is the so-called *uric acid diathesis*, in which condition there may be an habitual deposit of uric acid in the urine. It occurs also in febrile states with increased tissue meta-

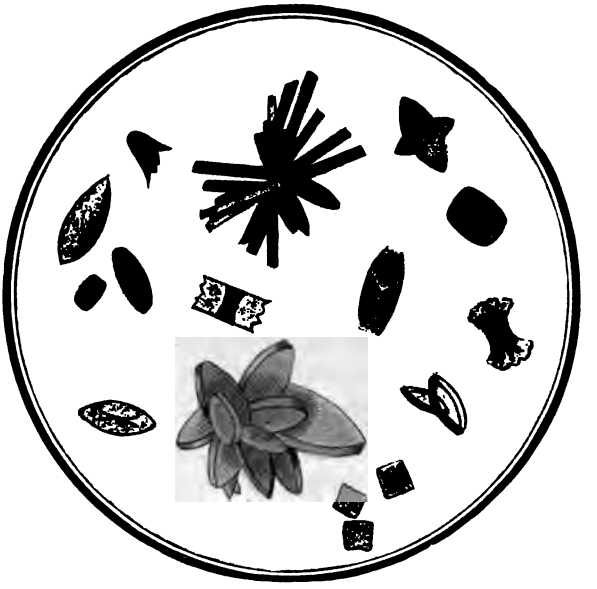

FIG. 107.—URIC ACID CRYSTALS.—(*Greene.*)

bolism and a decrease in the watery elements of the urine with a corresponding acidity.

Overindulgence in animal food, which is frequently the cause of interstitial nephritis, may be accompanied by the deposit of uric acid. On account of the fact that the urinary pigments tend to hold uric acid in solution, the polyuria accompanying the early stage of interstitial nephritis favors the formation of uric acid crystals.

Acid Urates.—Acid urates of *sodium*, *potassium*, *ammonium*, and *calcium* may be deposited in the urine. The mixed urate is common as amorphous particles which may remain suspended or settle to the bottom of the vessel. They may be easily recognized, as gentle heat quickly dissolves them, a property that is not possessed by any other urinary sediment.

Microscopically they appear as agglutinations of yellowish or pinkish granules and flakes.

FIG. 108.—SOME DEPOSITS IN ACID FERMENTATION OF THE URINE. *a*, Bacteria. *b*, Amorphous sodic urate. *c*, Uric acid. *d*, Calcium oxalate.—(*Landois.*)

Urate of sodium may be amorphous or form in fan-shaped or sheaf-like crystals.

Ammonium urate is usually found in alkaline urine that is undergoing fermentation. It may form in the urinary passages and produce serious results on account of the sharp spines setting up an intense irritation of the mucosa. It is a crystalline deposit consisting of spherules with projecting thorns, the so-called "hedgehog" crystals

At times these spines are very numerous and they may be curved and twisted.

Clinical Significance of Urates.—Besides the condition brought about by mechanical irritation by the spines of the ammonium urate crystals, the occurrence of urates is of little importance. The amorphous mixed urates are common in febrile diseases and all wasting diseases. They are also deposited during an attack of gout.

FIG. 109.—SOME DEPOSITS FROM AMMONIACAL URINE (ALKALINE FERMENTATION).

a, Acid ammonium urate. *b*, Ammonio-magnesium phosphate *c*, bacteria.—(*Landois.*)

Calcium Oxalate.—The most common form of calcium oxalate crystal is the *envelope* crystal.. They are small octahedral crystals with highly refractive diagonal planes giving the appearance of crossed diagonal lines.

With the low power they appear as bright specks, and it is only when viewed with the $\frac{1}{6}$ objective that their full beauty is revealed. Dumb-bell-shaped crystals sometimes occur, but are less frequent than the characteristic envelope form.

Calcium oxalate crystals are readily dissolved by the mineral acids, but are insoluble in water, acetic acid, alkalies, ether, and alcohol. Acetic acid forms a ready test to differentiate the short forms of triple phosphate from calcium oxalate, the former being quickly dissolved. Usually the microscopic appearance alone is sufficient.

FIG 110.—CALCIUM OXALATE, "ENVELOPE" CRYSTALS.—(*Greene.*)

Clinical Significance.—Usually little clinical significance is to be attached to a deposit of calcium oxalate. It occurs in neurasthenic states and in insanity quite frequently. Also after the ingestion of food rich in oxalic acid, notably asparagus, tomatoes, oranges, etc., and from excess of alkaline bases in the blood.

The amount of calcium oxalate present in the urine can be determined only by quantitative chemical analysis.

It is chiefly of interest in that it may form renal and vesical calculi. These crystals are more common in alkaline but may occur in faintly acid urines.

Ammonio-magnesium Phosphate.—This constitutes the so-called triple phosphates, and is the most common of all urinary crystalline deposits. Its most usual form is the *coffin-shaped* crystals, prismatic figures with beveled ends. These may be so short as to form squares, in which case they may be mistaken for envelope crystals of calcium

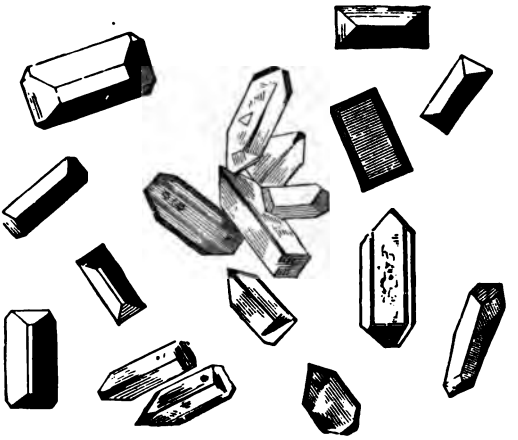

FIG. 111.—FORMS OF CRYSTALS OF AMMONIO-MAGNESIUM PHOSPHATE. —(*Tyson.*)

oxalate. More rarely beautiful fern-leaf or feathery crystals occur. These are usually formed when the urine is rendered very alkaline by the addition of ammonia. Various intermediate forms may be observed.

Calcium phosphate may occur in amorphous deposits as whitish flakes. It is precipitated by heat, and may therefore be mistaken for albumin. From this it can be distinguished by the addition of a few drops of nitric acid, which rapidly clears up the precipitate but does not

affect the albumin. The crystals of calcium phosphate are usually in the form of rosettes with radiating needle-like or wedge-shaped masses.

Basic magnesium phosphate is a rare deposit, occurring in alkaline or faintly acid urine in the form of highly refractive plates or rhombic tablets. They are quickly dissolved by acetic acid, and again precipitated on the addition of sodium carbonate.

According to Purdy, the following are the chief condi-

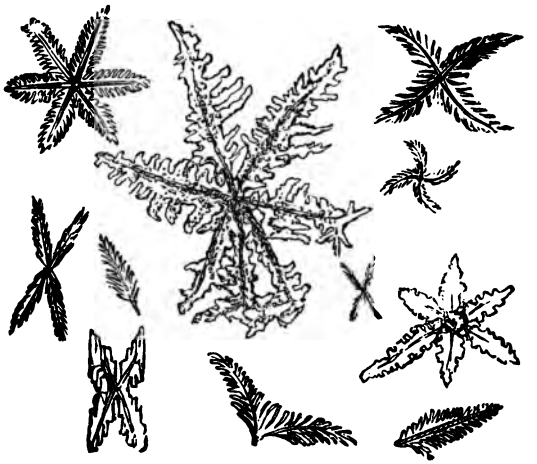

FIG. 112.—FEATHERY CRYSTALS OF TRIPLE PHOSPHATE.—(*Tyson.*)

tions of the urine which lead to the production of phosphatic sediments:

"(*a*) If the urine be alkaline from a fixed alkali.

"(*b*) If the earthy phosphates be in excess (the urine being alkaline or neutral).

"(*c*) If the urine be alkaline from volatile alkali, the result of decomposition of urea into ammonium carbonate in the urinary passages, the ammonium uniting with the

magnesium phosphate to form the triple phosphate of ammonium and magnesium."

Clinical Significance.—In cases of phosphaturia from the presence of a fixed alkali in the urine there is an alkaline reaction to the urine when voided, and symptoms of feeble respiration and general debility are often present.

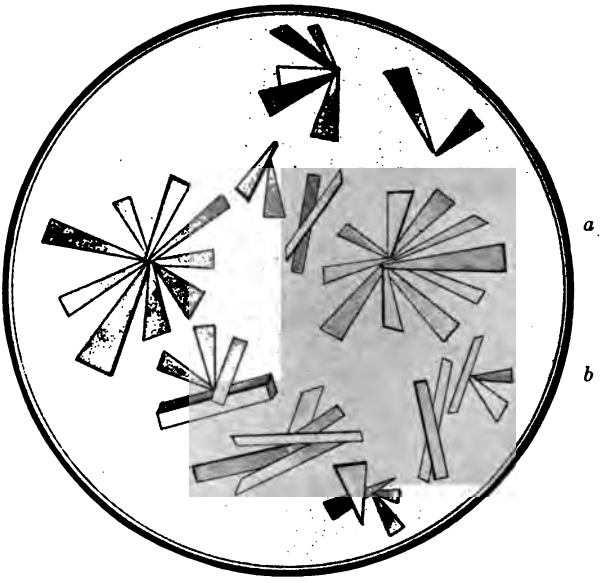

FIG. 113.

a, Calcium phosphate. *b*, Calcium sulphate.—(*Jakob.*)

This may be noted in the later stages of exhausting diseases and in flatulent dyspepsia. In the so-called "phosphatic diabetes," in which there is a large deposit of phosphates, the result of excessive elimination, severe symptoms of depression, irritability, and dyspepsia may be present.

When phosphaturia is due to the presence of a volatile

alkali, the urine undergoes ammoniacal decomposition before being voided, and the precipitation of phosphates takes place as soon as micturition is accomplished. Such a condition is always attended with an inflammatory lesion of some portion of the genito-urinary tract, and is therefore of a serious nature. Pus and mucus are present in addition to the phosphates.

This state of affairs is usually dependent upon retention

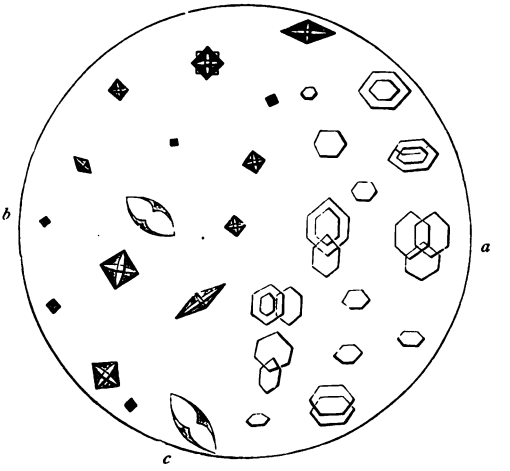

FIG. 114.

a, Crystals of cystin. *b*, Crystals of oxalate of lime. *c*, Hour-glass forms of *b*.

of urine on account of mechanical obstruction of the urinary channels. It may also depend upon nervous conditions favoring a retention of urine in the bladder.

Cystin.—Crystals of cystin occur in the urine as six-sided crystals or square prisms having a mother-of-pearl luster. If a drop of ammonia be added to the deposit on the slide, the cystin crystals quickly dissolve, to reappear when the

ammonia has evaporated. This is a rare deposit, devoid of clinical significance.

Tyrosin and Leucin.—These substances are chemically closely related, and result from the decomposition of proteids. They are exceedingly rare, occurring only in the most acute processes, notably in phosphorus-poisoning and acute yellow atrophy of the liver, though they have been found in severe cases of smallpox, enteric fever, and leukemia.

FIG. 115. — MAGNESIUM PHOSPHATE. —(*Gould.*)

Tyrosin forms sheaf-like bundles of fine needles. Leucin is seen as yellowish globular masses of high refractive power. From fat it is distinguished by its insolubility in ether.

Melanin.—Rarely there are deposited in the urine black

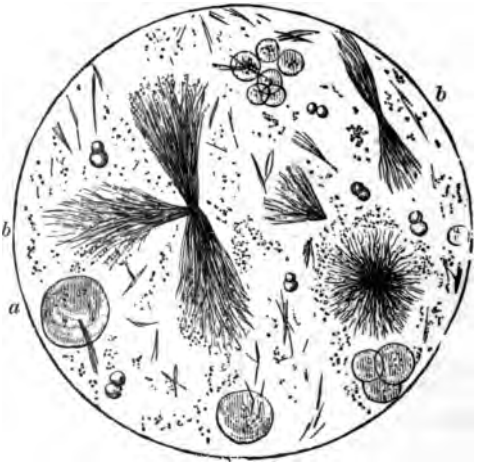

FIG. 116.

a, Leucin. *b*, Tyrosin.—(*Coplin.*)

or brown granular masses of pigment. They may occur in wasting diseases, and also in cases of pigmented tumors

of the urinary tract. From the former fact their presence cannot be accepted as indicating melanotic malignant disease, though when other symptoms are present they are a valuable confirmatory sign.

LIPURIA.

Fat in the urine occurs as globular masses of varying size which are highly refractile. It may be seen after fracture of the bones, in diabetes mellitus, and in acute degenerative renal processes associated with fatty degeneration of the tubular epithelium.

HEMOGLOBINURIA.

True hemoglobin in the urine is not an infrequent condition in those diseases that result in the destruction of the red blood-corpuscles in the vessels. Among these may be mentioned pyemia, poisoning by phosphorus, carbolic acid, scurvy, fat embolism, typhus fever, and malaria.

The urine is opaque, dark reddish-brown, and of high specific gravity, and there is a persistent pinkish foam. There may be a quantity of granular matter, and rarely some free blood and casts. If any doubt as to the nature of the coloring-matter exists, the micro-chemical test for blood will give the characteristic rhomboids of hemin in large numbers (see p. 288).

Organized Sediments.—Red Blood-cells: The red blood-cells may be present in the urine in quantity varying from a few cells, possible of detection only with the microscope, to a large amount. The color of the urine will not be affected unless they are present in considerable quantity, but in the latter case it may be bright red or dark brown, opaque, and frothy, the foam being pink. From pus-

corpuscles the red blood-cells are distinguished by their smaller size, pale yellow color, and the absence of granulations and nuclei.

In urine that is ammoniacal or of low specific gravity the red blood-corpuscles may become distorted and swollen. They then appear as more or less globular bodies of double contour, devoid of coloring-matter and scarcely visible even in a subdued light, or they may be seen as small rings whose outline is hardly distinguishable.

GUIDE TO THE ORIGIN OF BLOOD IN HEMATURIA.

ORIGIN.	QUANTITY.	DIFFERENTIAL POINTS.	MAY OCCUR IN:
Kidney.	Usually comparatively small.	Clots usually absent. Associated with blood-casts, epithelial and hyaline casts, renal epithelium. Intimately mixed with urine. Many swollen (loss of hemoglobin) phantom corpuscles. Sediment slight.	Acute and chronic nephritis. Malignant growths. Renal calculus, tuberculosis, embolism, abscess, acute febrile processes, hemophilia, and in filariasis and distomiasis. Frequently in poisoning from turpentine, carbolic acid, etc.
Pelvis of kidney and ureters.	Variable.	Absence of casts of any kind or renal epithelium. Fibrinous molds of ureters may be present. Pus-cells in calculus.	Disease of pelvis, calculus, etc.
Bladder.	Frequently large.	Blood-cells well preserved—unless urine is ammoniacal. Clots frequent. Heavy sediment. Pus in cystitis. In papilloma and malignant growths may be shreds of such tissue. If from neck of bladder, appears at end of micturition.	Stone, cystitis, tumors, varicose veins of vesical neck, etc.
Urethra.	Small.	May be expressed. First part of micturition.	Urethritis, trauma, etc.

At times they are shriveled and crenated, and it may be necessary to resort to the micro-chemical test for blood to determine its presence. In this case a few drops of urine are evaporated to dryness on a glass slip and the test proceeded with as already described (see p. 288).

The table on page 306 presents in concise form most of the important facts in relation to hematuria. In determining its origin it is of course necessary to take into consideration the history and general symptoms presented by the individual case. Even then the origin of the hemorrhage may not be fixed with certainty.

PYURIA.

Leukocytes.—White blood-corpuscles in small amount are found even in normal urine. Pus may be present in the urine in pathologic conditions in large quantity. It renders the freshly voided urine turbid, and white shreds and masses may generally be detected. Microscopically these will be seen to consist of strings and aggregations of pus-corpuscles. When allowed to stand for some hours, urine containing pus in quantity presents a dense, viscid, whitish precipitate.

FIG. 117.—PUS IN THE URINE.
Many are irregular or fragment forms. —(*Gould.*)

Pus-corpuscles in the urine are seen under the microscope as more or less spherical, coarsely granular bodies, somewhat larger than a red blood-cell and possessing one or more nuclei. If a drop of acetic acid be added to the drop of urine, the granulations of the protoplasm of the pus-corpuscles disappear and the nuclei are brought strongly into view. After a little practice pus is easily distinguishable even without the aid of this reagent.

By drying a drop of urine on the slide and fixing by heat, the slide being warmed until slightly uncomfortable to the fingers, the pus-corpuscles may be stained with methylene-blue, which brings out the characteristic nuclei.

Pus is the most common of urinary sediments, and may come from any portion of the genito-urinary tract. A large abscess breaking into some part of the genito-urinary tract may produce sudden pyuria with a heavy deposit of pus-corpuscles.

It is always important when pus appears in the urine to determine its exact source, but this may be a matter of extreme difficulty. All the facts of the case must be borne in mind, and in doubtful cases several examinations should be made before reaching a conclusion.

While the statements in the following table are not absolute, they may serve as an indication of the probable origin of the pyuria.

GUIDE TO ORIGIN OF PUS IN PYURIA.

Disease.	Reaction.	Urinary Sediments.
Renal Congestion.	Acid.	Pus in very small quantity. A few red blood-cells and hyaline casts.
Nephritis.	Acid.	Pus usually in small amount, being greatest in acute disorders. Also present renal epithelium, tube-casts, and perhaps blood.
Pyelitis.	Acid.	Quantity of pus may be large. Epithelium from pelvis of kidney. In uncomplicated cases no tube-casts. May be sudden disappearance and return of pus owing to stoppage of ureter upon the affected side.
Cystitis.	Alkaline.	Pus in small or large amounts. Cells may be disintegrated, a stringy, mucoid substance being voided. Cells from deep layer of mucosa of bladder in severe cases. Absence of renal cells and casts.
Urethritis.	Acid.	Usually gonorrheal.
Vaginitis.	Acid.	Pus and vaginal cells.

The genito-urinary tract is lined with a mucosa the epithelial characteristics of which differ very widely according to the location and purpose of the part. Certain portions are secretory in function, and from these a variety of glandular epithelium may find its way into the urine. Other parts are merely passageways, or, as in the case of the bladder, temporary receptacles for the urine

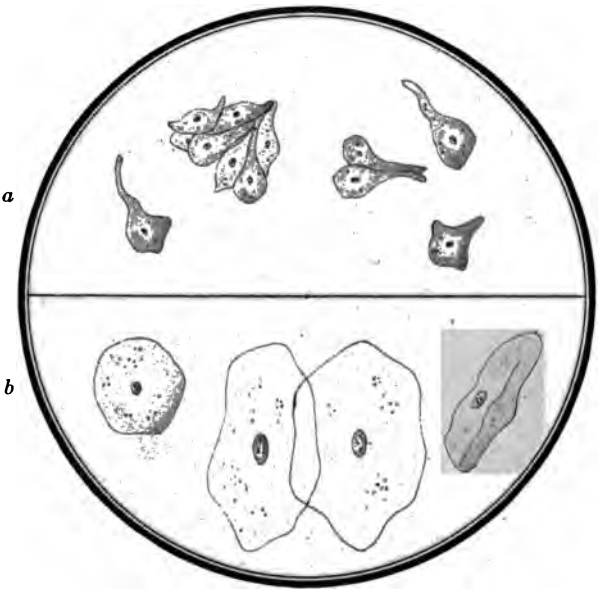

FIG. 118.

a, Epithelium from renal pelvis. *b*, Vaginal epithelium.—(*Greene.*)

and other fluids, and here are found coverings of cells the upper layers of which are more or less flattened and subject to constant renewal, the lower strata consisting of cells of a variety of shapes, the whole constituting stratified eptihelium.

From these facts it will be readily seen that the urine may contain epithelial elements of great diversity of

form, and it would seem upon superficial inspection of the subject to be an easy matter to determine their origin. This, however, is not the case, and unless there exist other factors to guide us it may frequently be quite impossible to determine with exactness the origin of epithelium found in urinary sediment. For it must be remembered that detached and single cells from points widely separated may present a similar appearance, and also that such cells

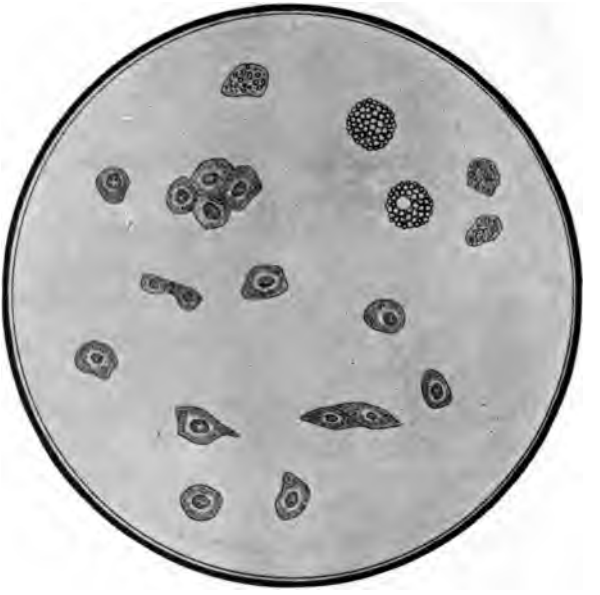

FIG. 119.—VARIOUS FORMS OF RENAL CELLS.—(*Greene.*)

frequently take up watery or other elements from the urine and become swollen and distorted. Their characteristics may also be obscured by masses of bacteria which have formed in them after the urine has been passed, or, in the case of cystitis or pyelitis, even while the cells were still within the body.

In determining the origin of epithelial cells in the urine

one should have constantly in mind the histology of the genito-urinary tract, and he must also be in great part guided by the occurrence or absence of other deposits that

FIG. 120.—BLADDER EPITHELIUM.
a, Surface. *b*, Deeper layers.—(*Greene.*)

frequently may throw light on the subject. The distinguishing differences between pus and epithelium are as follows:

PUS.	EPITHELIUM.
1. Cells globular.	1. Cells globular or otherwise.
2. Protoplasm coarsely granular.	2. Protoplasm finely granular.
3. Nucleus usually indistinct.	3. Nucleus usually indistinct.
4. Nucleus brought out by acetic acid.	4. Nucleus usually single and often large.
5. Nucleus usually multiple (polynuclear leukocytes).	5. Cells frequently contain fat-droplets.
6. Most frequent cellular deposit.	6. May occur in masses when character of tissue can be seen.
7. Often in large quantity.	7. Usually small in amount.

Modern writers classify the epithelium of the genito-urinary tract into:

SQUAMOUS EPITHELIUM.*

FORM.	ORIGIN.
Flat, irregular outline. Small, distinct, and usually central nucleus. Slightly granular protoplasm.	Prepuce, vulva, vagina, and bladder.

COLUMNAR EPITHELIUM.

FORM.	ORIGIN.
Polyhedral, spindle, or caudate. May show indentations from recent contact with neighboring cells. Often in groups. Nucleus prominent without acetic acid.	Superficial layer of renal pelvis. Deep layers of bladder. Ureters or urethra.

SPHEROIDAL EPITHELIUM.

FORM.	ORIGIN.
Small, round, or irregular, finely granular, often contain *fat-droplets*. Nucleus comparatively large and prominent, usually nucleolus, may occur in groups. Frequent upon tube-casts, forming the "epithelial" cast.	Usually convoluted tubules of the kidney. May come from deep layers of mucosa of renal pelvis, bladder, and urethra.

Epithelial elements from the parenchyma of the kidneys are thrown off only under pathologic conditions of those organs. When they occur in any quantity, they are always associated with other elements that lead to an easy diagnosis of the condition—tube-casts and albumin being present in all serious renal troubles at one or another time of the disease.

* The table is taken from Purdy's "Urinalysis."

URINARY CASTS.

The multiplicity of terms by which these bodies are known is often confusing to the student, and yet the subject is exceedingly simple. True tube-casts are formed by the collection in the lumina of the uriniferous tubules of a coagulable substance, probably derived from the blood. This collects in fluid form, and upon coagulation shrinks slightly and escapes from the tubule and is cast off with the urine. This then gives us our true hyaline cast. Most other forms are but modifications of this one.

Fragments of tubular epithelium or entire desquamated cells, red blood-corpuscles, pus, fat, or bacteria may adhere to and become embedded in the hyaline cast, thus forming a body which is designated by the name of the constituent predominating. The exact pathologic process that was present in the individual tubule in which the cast was moulded at the time of its formation is graphically set forth by the nature of the cast itself.

The following table is intended to portray the mode of the formation of the varieties of true tube-casts and the pathologic condition of the tubule necessarily present in each case.

Central Core.	Special Pathologic Condition of Tubule when Cast was Found.	Result.
Coagulation of albuminous material from the blood.	Integrity of tubular epithelium not affected.	Hyaline casts.
Hyaline cast plus:	Granules from disintegration of tubular epithelium.	Granular cast.
" " "	Epithelial cells, desquamated from inclosing tubule.	Epithelial cast.
" " "	Pus-cells, suppurative process in the tubule.	Pus cast.
" " "	Fat, from fatty degeneration of tubular epithelium.	Fat cast.
" " "	Bacteria, from invasion of the tubule by these organisms.	Bacterial casts.

True tube-casts may be readily differentiated from the pseudo-casts by the addition to the urine of a drop of dilute acetic acid, which quickly dissolves the central core and liberates the bodies embedded in it. Pseudo- and mucous casts are unaffected by acetic acid.

Hyaline Casts.—These bodies are of varying diameter,

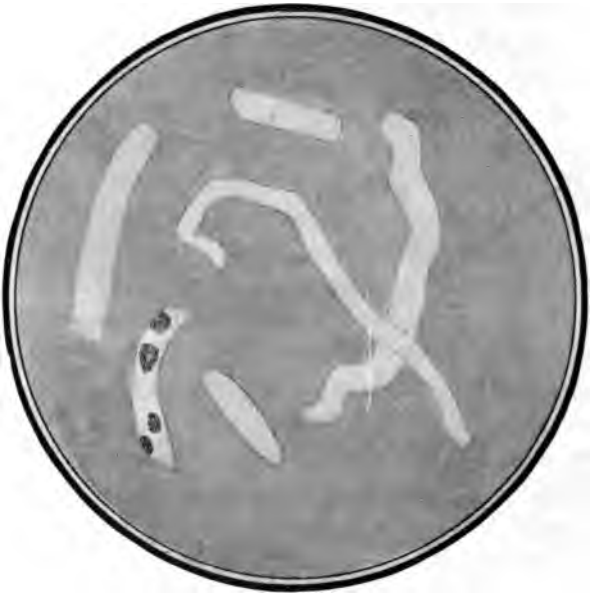

FIG. 121.—SHOWS THE EFFECT OF LOW ILLUMINATION, THE HYALINE CASTS BEING PLAINLY VISIBLE.

conforming to the caliber of the tube in which they were formed. They usually are slightly curved and one or both ends are rounded. They may be almost colorless or from a pale yellow to a reddish-brown.

Granular Casts.—Purely hyaline casts are seldom seen. They usually contain fine or coarse granules, the products of cellular necrosis, in greater or less abundance.

Epithelial Casts.—In addition to the above granules, renal epithelium, either entire or necrotic and fatty, may be embedded in the hyaline matrix. The cells may be few in number or in sufficient quantity to obscure the central core of hyaline substance.

Pus Casts.—The hyaline cylinder may be covered with

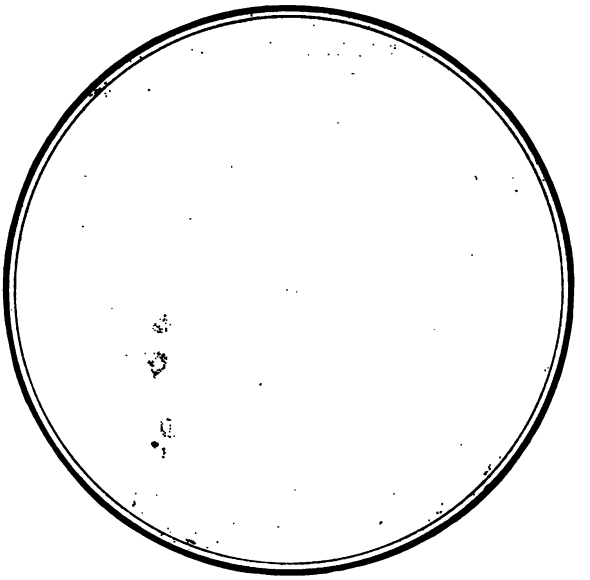

FIG. 122.—SHOWS THE EFFECT OF HIGH ILLUMINATION, THE HYALINE CASTS BEING LOST IN THE FLOOD OF LIGHT, AND ONLY THE RENAL CELLS APPEAR.—(*Greene.*)

leukocytes. More frequently there are granules, pus, and epithelial cells present in the same specimen.

Fatty Casts.—While casts presenting a few fat-droplets in conjunction with other elements are frequently seen, there sometimes occur hyaline cylinders that are completely covered by fat-globules of varying size. They are highly refractile bodies and indicate an exquisite degree of

fatty degeneration of the renal epithelium. The addition of ether quickly dissolves the fat-droplets and furnishes a ready means of diagnosis.

Bacterial Casts.—When the hyaline core is thickly covered with micro-organisms, it may readily be mistaken for a true granular cast. From the latter it may be distinguished by the more even distribution and size of the granulations and by examination with the high power, which will usually reveal their true nature. They are also quite resistant to the strong mineral acids and caustic alkalies. Their presence is of great import, signifying a suppurative nephritis or pyelitis.

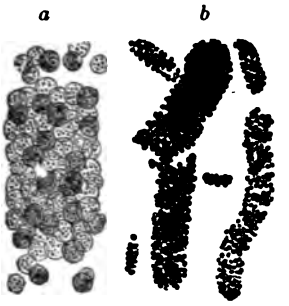

FIG. 123.

a, Cast made up almost purely of leukocytes. *b*, Cast composed of acid sodic urate crystals.—(*Landois.*)

Pseudo-casts differ from the true casts in their mode of formation and physical properties. They are of two kinds, the first consisting of a cylinder of epithelial cells that have desquamated *en masse* from a uriniferous tubule. This form is hollow and is of rare occurrence, being found chiefly in severe cases of desquamative nephritis. The second variety is composed of blood that has escaped into a tubule and coagulated there. It consists, therefore, of fibrin, and is thickly studded with red blood-corpuscles, with perhaps here and there a leukocyte. The latter variety is indicative of renal hemorrhage.

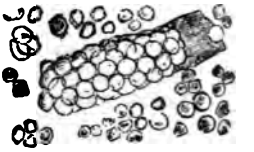

FIG. 124.—BLOOD-CELLS AND BLOOD-CAST.—(*Landois.*)

Cylindroids are hyaline casts of extreme length and

irregular conformation. They vary in thickness in different portions of their extent, and may be indented, wavy, and ribbon-like. They frequently are twisted or folded upon themselves, and may divide into tail-like processes. Sometimes they contain crystals, and rarely epithelial cells.

Their exact significance is not established. They are

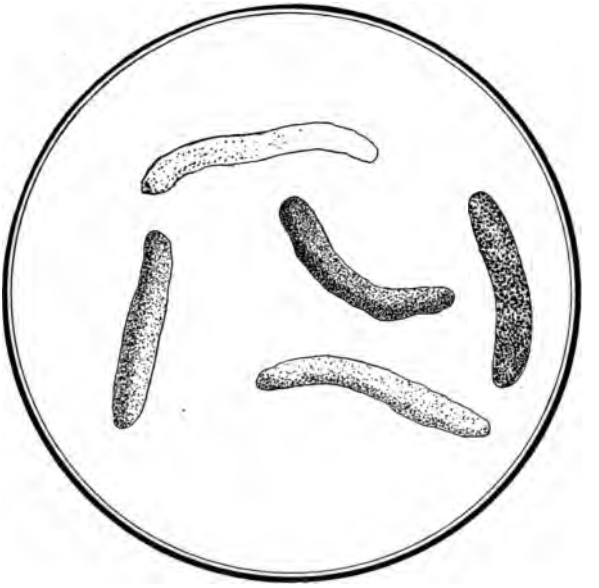

FIG. 125.—GRANULAR CASTS.—(*Greene.*)

dissolved by acetic acid, and as they occur in renal congestion and in various forms of nephritis in connection with true casts, their presence in the urine is sufficient to cause apprehension. On the other hand, it is claimed that they have been found in cystitis and in apparently normal urine.

Waxy Casts.—This name has been given certain casts of

uncertain origin and peculiar characteristics. They may be long and slender, but are usually short, broad, and indented. They are highly refractile and resist the action of acetic acid. They have been thought to be of an amyloid nature, but may occur in conditions other than amyloid kidney and may be absent in this affection. The

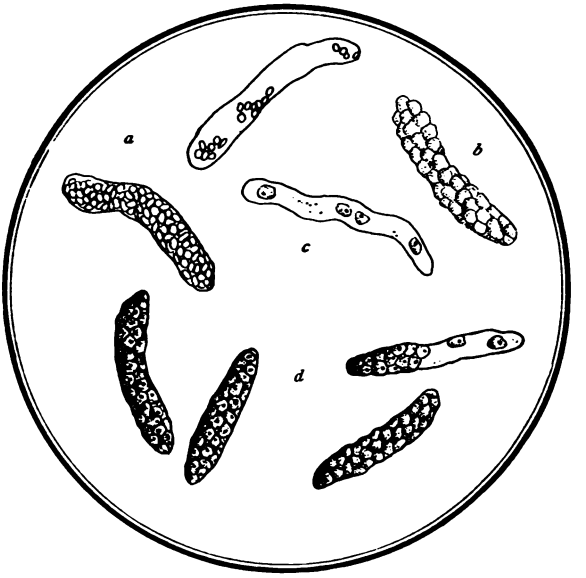

FIG. 126.

a, Blood-cast and hyaline cast carrying blood-cells. *b*, Pus cast. *c*, Hyaline cast carrying epithelial cells. *d*, Epithelial cast.—(*Greene.*)

theory that they are hyaline casts which have remained a long time *in situ* and undergone chemical change has been advanced, and is plausible.

Crystalline Casts.—Rarely there occur casts of urates or hematoidin crystals. They are of little consequence from a pathologic point of view, and occur chiefly in the urine

of infants, and sometimes in cases of gout or renal congestion.

Mucus-cylinders.—These may be seen in any specimen of urine containing mucus. They are long, flat, twisted, hyaline bodies of varying diameter. Frequently they divide into tapering processes. They are unaffected by

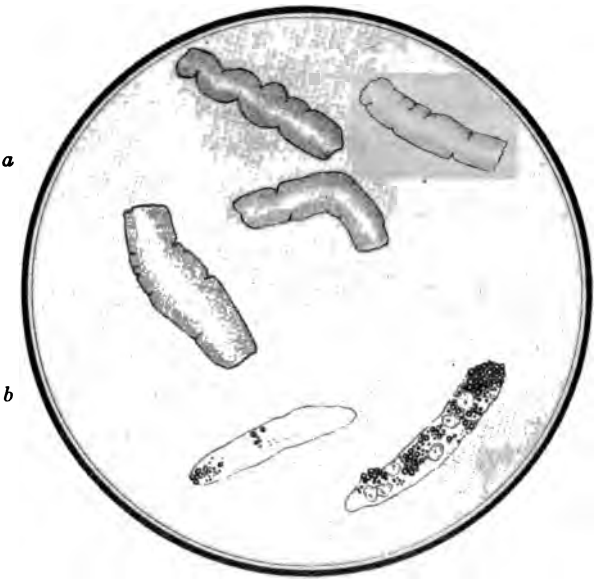

FIG. 127.

a, Waxy casts. *b*, Fatty casts.—(*Greene.*)

acetic acid. No practical significance is to be attached to them.

Attention is again called to the fact that in searching for tube-casts a very subdued and preferably an oblique light must be used. Strong light shines *through* hyaline and granular casts and renders them invisible.

The special significance of the various forms of casts

will be considered under the discussion of nephritis. True casts are never present in normal urine except in small amount, and then are of the hyaline variety and are transient. They may occur, as above, after severe muscular exertion or subjection to cold, and indicate a temporary congestion of the kidney. Their persistence and the presence of albumin are to be taken as indications of renal disease.

The importance of tube-casts as an aid in the diagnosis of kidney lesions cannot be overestimated, and their presence in the urine should under all circumstances lead to a further investigation, even though they may be temporarily accounted for by the patient having recently undergone severe muscular effort or exposure.

Spermatozoa.—The description of these bodies will be found in the section on semen. Spermatozoa may occur in normal urine of the male after coitus or masturbation and nocturnal emissions or those due to muscular spasm in epilepsy. The semen may also be voided in the act of defecation, being pressed from the seminal vesicles by the passage of hard feces.

In *spermatorrhea* they appear in the urine in considerable quantities, frequently rendering it turbid and forming a whitish sediment. Sediments consisting of phosphates are frequently mistaken by the laity for spermatic deposits. A microscopic examination readily determines the presence of spermatozoa, for they can be mistaken for nothing else by even the unpractised eye.

Spermatozoa may occur in the urine of women after coitus.

Fragments of Neoplasms.—In the villous form of papilloma of the bladder shreds of the tumor are frequently voided in the urine and are easily recognized under the microscope.

If doubt as to their nature exists, they should be embedded in celloidin, cut, and stained after the usual method. Single cells from neoplasms of the genito-urinary tract afford little positive information, and usually the presence of renal neoplasms cannot be diagnosticated by microscopic examination of the urine. Particularly in the female, where the urethra is short and wide, polypi of considerable size may pass entire.

The presence of necrotic masses of tissue containing pigment points to melanotic carcinoma of some portion of the urinary tract, particularly if associated with hematuria, pain, and cachexia.

Bacteriuria.—Normal urine is sterile until it leaves the bladder. If, therefore, micro-organisms in quantity are found in urine immediately after it is voided, the fact of infection of some portion of the urinary tract is established. To make doubly sure that the organisms do not come from the external genitals, the urine may be drawn with a sterile catheter, the external parts having been previously cleansed, and in cases of a known infection of the urethra that part thoroughly irrigated with a weak permanganate of potash solution.

All urine after standing for even a short time contains bacteria which have accumulated after its exit from the bladder, for urine is a very good culture-medium, permitting of rapid multiplication of fungi. This is particularly true of diabetic urine, in which all of the lower vegetable forms flourish.

Two classes of micro-organisms are found in urine—pathogenic and non-pathogenic.

Non-pathogenic Fungi.—These may be either *moulds, yeasts, or bacteria.*

Moulds are seldom seen, and are of little importance.

Yeasts occur chiefly in urine containing sugar. They

are to be distinguished from epithelium by their smaller size and the fact that they present budding processes and frequently form bead-like masses. Many of the more adult forms possess a nucleus. They are usually oval in form. Care must be exercised in order to distinguish the non-nucleated cells from red blood-corpuscles.

Bacterium termo and other putrefactive organisms are frequently met with in cases of cystitis with ammoniacal urine and in the urine of old and enfeebled persons. Even though the organisms may be non-pathogenic, this is a serious condition on account of the decomposition of the urine *in situ* which is occasioned by their presence.

Putrefactive organisms include all forms of bacteria—bacilli, cocci, and spirilla. They are frequently actively mobile, and beautiful examples of spiral forms in active motion may be seen in almost any specimen of urine that has been allowed to stand for a few days until it has undergone ammoniacal fermentation.

Pathogenic Fungi.—It has been well demonstrated that in the effort to eliminate bacteria which have gained entrance to the circulation the kidneys or bladder may become affected. The pyogenic organisms are the ones most frequently found in renal lesions, and, consequent upon this, in the urine. Chief among these are the staphylococci and the streptococci.

In enteric fever typhoid bacilli are found in the urine in a large percentage of cases, even in the early stages of the disease.

Tuberculosis of the kidneys is quite common as a secondary affection following pulmonary tuberculosis. The bacilli may occur in the urine in large numbers. If such is the case, they are often found in S-shaped clumps. For the method of detecting the tubercle bacillus in urine and its differentiation from the smegma bacillus the reader is

referred to the section on tuberculosis. In doubtful cases a guinea-pig should be inoculated with a quantity of the sediment obtained under precautions as to cleanliness. Cultural methods recommended by some text-books are extremely difficult, and therefore of little value.

Actinomyces have been found in the urine in cases where the genito-urinary tract was infected by this fungus.

The *Gonococcus of Neisser* is frequently found in the pus-corpuscles in urine from cases of gonorrhea. This subject is treated at length in the consideration of the gonococcus.

VERMES IN THE URINE.

Distoma hæmatobium.—As mentioned in the section on blood, the eggs of this parasite are frequently found in the urine of persons whose blood is infested with the worm. Their sharp spines cause an intense irritation of the mucous membranes of the bladder and urethra, and micturition is accompanied with pain and hemorrhage. The disease is limited to the tropics, but is occasionally seen in sailors and others just returning from these parts.

The eggs should be sought for in urine of suspected cases, and are readily detected and identified by their peculiar form and spines. The urine also frequently contains pus and blood.

Filaria sanguinis hominis.—This worm, whose normal habitat is the blood, is at times found in the urine of persons infested with it.

Echinococci.—If the contents of an echinococcus cyst burst into some part of the urinary tract, the characteristic hooklets and scolices may appear in the urine. Primary involvement of the urinary passages is rare.

Infusoria.—The novice may be puzzled by the detection in the urine of actively motile cellular organisms, the like

TABULATION OF RENAL DISEASES AND MICROSCOPIC APPEARANCES OF URINARY SEDIMENTS IN EACH.

Disease.	Reaction.	Specific Gravity.	Amount of Urine.	Albumin.	Sediments.
Acute hyperemia, .	Acid.	Slightly reduced.	At first increased.	Present in small amount.	Red blood-cells. May be a few hyaline or granular casts and tubular epithelium.
Passive hyperemia,	Acid.	Increased 1.025 to 1.030.	Diminished.	Small amount	Amorphous urates and uric acid crystals, a few hyaline casts, and perhaps renal epithelial cells.
Acute diffuse nephritis, ...	Acid.	Varies with amount of urine passed.	Diminished. May be suppression.	Large quantities.	Red blood-cells (often crenated and swollen). Renal epithelium in quantity. Blood-casts, hyaline, pus, and epithelial casts.
Chronic diffuse nephritis, ...	Acid.	Usually low.	Usually diminished. Later stages increased.	Usually in large quantities.	Usually abundant. Casts in quantity, both hyaline, granular, and epithelial. Sometimes fatty casts are observed. Pus and fatty renal epithelium in large amount. Granular casts predominate and are large and broad. Granular detritus. Red blood-cells rarely present.
Chronic interstitial nephritis,	Acid.	Usually low.	Increased, sometimes largely. In later stages diminished.	Intermittent.	Usually very scanty. A few hyaline casts at intermittent periods. Renal epithelium rarely present. Uric acid and calcium oxalate crystals.
Amyloid kidney, ..	Acid.	Decreased; may be 1.006.	Increased.	Present in large amount.	Very scanty. A few hyaline casts. May be waxy casts.
Cystic kidney, ...	Acid.	1.010 to 1.015.	Increased.	May be in quantity.	Usually large granular casts. Usually hematuria. Small amount of pus. Phosphates increased. Triple phosphate crystals.

Renal tuberculosis,	At first acid. Later alkaline.	Lowered.	Increased.	Small amount	Early stages a few red blood-cells. Later pus in varying and increasing amounts. May be marked hematuria. Mucous, caseous masses and renal detritus. Tubercle bacilli may usually be demonstrated.
Renal cancer,.....	At first acid.	Lowered	Increased at first.	Usually present. Always, if there is blood.	Red blood-corpuscles in quantity. May be pus. Perhaps masses of the neoplasm.
Surgical kidney,...	Acid if limited to kidney.	1.016 to 1.006.	Reduced.	Variable in quantity.	Large amount. Pus, blood, renal detritus. Usually casts. Bacteria and bacterial casts.
Chronic pyelitis, ..	Faintly acid.	Decreased.	Increased.	In small quantity.	Pus may form in plugs in papillary ducts. Epithelium in small amount and not characteristic. May be caudate and spindle cells. Perhaps triple phosphate.

of which perhaps he has never seen. They will probably prove to be infusoria that have gained access to the urine through the vessel in which it was collected having been washed in water inhabited by them. The author has seen such cases a number of times. In one of these vorticellæ in abundance and of great activity were found, while in several other instances *rotifers* were observed.

Diatomes and algæ are frequently met with. The microscopist should familiarize himself with these various forms by the study of pond- and ditch-water, in which they abound. That interesting little volume "Ponds and Ditches," by M. C. Cook, may serve as a guide to any so inclined.

OTHER FOREIGN BODIES.

Besides the above, a great variety of foreign bodies may gain access to the urine, either through the use of unclean vessels or from the dust floating in the air of the room. Among the most common are the fibers of cotton, wool, silk, and hemp, feathers, vegetable matter, hairs, and small seeds.

Fecal matter may also be present, either from the patient having defecated in the receptacle in which the urine was collected or having voluntarily introduced it with the purpose to deceive or through a vesico-rectal fistula.

Hysterical patients may introduce foreign bodies into the urethra or vagina, or they may be shown the physician as having been voided with the urine.

CYSTITIS.

Pus and mucus in varying quantity, with red blood-cells in some instances, and bacteria in enormous numbers in the freshly voided urine are the chief microscopic characteristics of cystitis. Vesical epithelium is also present, sometimes in large quantity.

This disease in its various forms is always due to a bacteriologic infection of the bladder by pyogenic organisms. In conjunction with the history and clinical symptoms, this condition of the urine renders diagnosis a matter of no difficulty. Owing to the ammoniacal decomposition of the urine *in the bladder*, triple phosphate and ammonium urate in the form of hedgehog crystals may be seen. The author has observed one case in which the latter crystals were present in such quantity as to form a heavy deposit.

VESICAL CALCULUS.

In the early stages the frequent presence of crystals of uric acid or oxalate of calcium may, in conjunction with the clinical features, aid in the diagnosis of stone. As the disease progresses infection of the injured vesicular mucosa always results, and to the previous condition cystitis is added. Hematuria of intermittent character is the rule.

VESICAL TUBERCULOSIS.

From the first there is pus in the urine in gradually increasing amount. The cystitis that is set up must be differentiated from other varieties by the clinical symptoms and by examination of the sediment for the tubercle bacillus (see p. 177).

THE SEMEN.

The semen as ejaculated consists of the combined secretions of the testicles, vasa deferentia, vesiculæ seminales,

the prostate and Cowper's glands, and the mucus-glands of the urethra. The element of chief importance in this rather complex fluid is the spermatozoon.

Spermatozoa are the vitalizing principle of the semen. They are derived from the spermatogenetic cells of the testicles and are elongated bodies (about 50 μ) consisting of a thread-like tail, a middle piece, and a head. The latter is about 3 μ to 5 μ in length, oval, and flattened, the sharper end presenting anteriorly.

In normal semen and in the secretions of the vagina these bodies have the power of active motion, being propelled by undulating and rapid movements of the tail. In the vagina they may live for days and weeks. In the seminal fluid they may retain their vitality for a considerable length of time provided they be kept at body-temperature and evaporation guarded against.

Besides spermatozoa, the semen contains epithelial cells of various kinds, and if it is allowed to stand for some time these form crystals that chemically and in their shape do not differ from the Charcot-Leyden crystals found in the sputum. They are known as spermatic crystals. According to von Jaksch, they are deposited in large numbers when a 1 per cent. solution of ammonium phosphate is added to pure prostatic fluid, and their appearance in quantity in the semen is an indication of prostatorrhea.

Not infrequently the physician is called upon to determine the cause of sterility in married couples. Kehver found that in a series of forty cases of sterile marriages the spermatozoa were absent from the semen of the husband in fourteen. The spermatozoa may be present and the cause of sterility be due to their lack of motility. In such instances it will be found that in a short time after ejaculation they become entirely motionless.

The semen should be examined as soon after copula-

tion as possible, a drop being placed upon the slide together with a drop of normal salt solution (0.6 per cent.) and covered with a cover-glass to prevent evaporation. The $\frac{1}{6}$ objective should be used.

MEDICOLEGAL EXAMINATION FOR SPERMATOZOA.

In Rape Cases.—Interesting and important facts may be gleaned from microscopic examination of stains upon the clothing, deposits or crusts about the external genitals, and scrapings from the vagina. As has been mentioned, the spermatozoa may retain their vitality in the vagina for many days.

Scrapings should be made and the product diluted with normal salt solution and examined with the high power It has also been frequently shown that spermatozoa resist drying and putrefaction, and Roussin reported a case in which they were detected in the stains upon a piece of cloth polluted eighteen years previously.

The suspected seminal stains should be soaked in normal salt solution for some hours and then squeezed out upon a glass slip. Many spermatozoa, whole or in part will be found if the stain is due to semen.

The detection may be facilitated by the addition to the normal salt solution, in which the stain is moistened, of enough aqueous solution of eosin to impart to it a pink color. By this means the spermatozoa will be stained, the head becoming quite red and the tail a delicate pink. Usually this is unnecessary, as the spermatozoa are easily recognized in the uncolored condition.

Their absence from the stain does not necessarily prove the same to have been made by some substance other than semen, as the man may have been impotent. In a case

where spermatazoa cannot be found, the stain should be subjected to the chemical test for semen devised by Florence.

According to this author, dark brown crystals of iodospermin are formed when semen or a dilution of seminal stains is treated with a solution of iodo-potassic iodid after the following formula:

Iodin crystals,	1.65
Potassium iodid,	2.54
Water,	26.00

The crystals occur in the form of brown needles, singly,

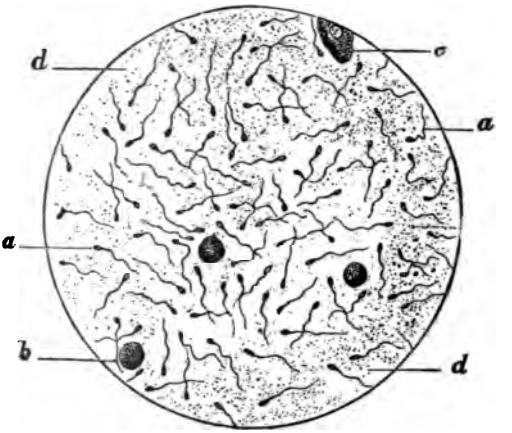

FIG. 128.—SEMINAL ELEMENTS, SOME OF WHICH MAY BE FOUND IN THE URINE.

a, *a*, Spermatozoa. *b*, Seminal cells. *c*, Epithelium. *d*, *d*, Seminal granules.—(*Coplin.*)

in pairs, or in the form of rosettes or in long rhombic plates. They are readily recognized under the microscope. Examination should immediately follow the application of the reagent, as the crystals are unstable and may redissolve.

It goes without saying that in rape cases a careful note should be made of all other substances detected microscopically, such as hairs, fibers of various kinds, epithelial cells, and blood-corpuscles, as these may materially aid in the true solution of the case.

VAGINAL SECRETIONS.

The normal vaginal secretion is a thin fluid of acid reaction containing mucus and many large pavement epithelium cells and mucus-corpuscles.

Bacteria of various kinds are also present, among them a diplococcus that decolorizes by Gram's method and resembles the gonococcus morphologically. For this reason Simon considers it necessary that cultures should be made before reaching a conclusion that gonorrhea is present in the case of married females and children. This precaution is, of course, necessary only in selected cases.

CEREBROSPINAL FLUID.

There are a number of conditions in which an examination of the cerebrospinal fluid is of great importance.

In order to obtain this fluid lumbar puncture must be resorted to. Briefly, this procedure is carried out as follows.

Strict surgical cleanliness is necessary for the sake of the

patient, and also that, if it is deemed necessary, cultures may be made. In the latter instance large quantities of the fluid must be used for inoculation, as otherwise no growth may result owing to the organisms being present in small numbers.

The skin of the patient having been prepared for surgical procedure, he is placed upon the right side, with the knees flexed and the left shoulder depressed. A long, thin aspirating needle is then introduced 1 cm. to the right of the median line between the spines of the third and fourth lumbar vertebræ, and passed upward and inward to the depth of 3 to 4 cm. in children, or 7 to 8 cm. in adults.

As soon as the point of the needle enters the subarachnoid space cerebrospinal fluid flows from it in drops. The fluid may be allowed to run directly into the culture-tubes and a small quantity be collected in a sterile test-tube for microscopic examination.

It is scarcely necessary to add that the needle with which the puncture is made and the hands of the operator must be sterilized as if for a laparotomy.

Pathogenic organisms may be detected in the exudate microscopically. It is advisable to centrifuge it and examine the bottom strata in the tube; or, if a centrifuge is not available, to place the tube of exudate in the ice-chest for a few hours so that bacteria and pus-cells may settle. The meningococcus, tubercle bacillus, pneumococcus, and many other organisms have been found in the cerebrospinal fluid.

Methylene-blue is usually a good stain for routine examination, but, of course, if the presence of the tubercle bacillus is suspected, it is necessary to follow the method laid down for the staining of this bacterium.

Descriptions of the meningococcus and other bacteria

that may be found in cerebrospinal fluid will be found in the section on bacteriology.

NASAL SECRETIONS.

The nasal fossæ are lined with a mucous membrane covered with stratified columnar epithelium and containing numerous mucous and serous glands. These glands furnish an excretion which is transparent and tenacious, and alkaline in reaction. It consists largely of columnar epithelium, together with goblet cells and leukocytes.

Large numbers of bacteria are always present in nasal secretions. Tubercle bacilli, Bacillus mallei, diphtheria bacilli, and the thrush fungus may be found accompanying the lesions in the nasal cavity due to their presence. Diplococcus intracellularis meningitidis has been found in the nasal secretions, and this may account for those cases of meningitis following traumatism affecting the integrity of the brain-cavity. In catarrhal conditions of the nasal membranes the desquamated cells may be found in large numbers. Pustular discharges may contain any of the pyogenic organisms.

SALIVA.

The normal secretion of the salivary glands of the mouth is a clear, somewhat viscid fluid of alkaline reaction. It contains, in various amounts:

1. Salivary corpuscles, which are bodies resembling the

leukocytes of the blood, except that they are larger and have a very granular protoplasm.

2. A few red blood-corpuscles may be found.

3. Epithelial cells from the mucous membrane of the mouth.

When from the surface, these cells are large, flat plates with irregular borders and a small nucleus. If they are derived from the deeper layers of the mucous membrane, they are polyhedral in form with large nuclei.

Bacteria.—Over fifty distinct bacteria have been cultivated from the normal saliva. Many of these are capable of becoming pathogenic under favorable circumstances. Among them are the pneumococcus, streptococcus, staphylococci, and diphtheria bacillus.

The tubercle bacillus may be demonstrated in tubercular lesions of the buccal cavity or respiratory passages, and it is claimed that it may sometimes be present even under normal conditions. The causal agents of actinomycosis, thrush, and gonorrheal stomatitis are treated elsewhere.

Leptothrix buccalis.—This is a large organism occurring in threads in which it is difficult to demonstrate segments. It is devoid of pathologic significance, but is frequently found in the sputum of various pulmonary diseases.

SPUTUM.

By the term sputum is meant those substances that are discharged from the lungs and air-passages through the act of coughing or that rise spontaneously and are expectorated. Dependent upon whether the air-passages are normal, or upon the form and character of the patho-

logic process, the quantity and composition of the sputum may vary greatly.

The sputum may be clear, thin, and viscid, or thick and colored by blood, pus, or bacteriologic pigments. Bacillus pyocyaneus frequently imparts a greenish hue to sputum in which it occurs.

In abscess of the lung, empyema, or gangrene the odor of the sputum may be very offensive.

Microscopic Examination of Sputum.—For the general inspection of sputum it is advisable to place a quantity of the collected material in a large Petri dish. It may then be viewed first by transmitted light; then, the dish being placed on a black surface, or a black piece of paper, objects such as cheesy masses, elastic tissue, and fragments of lung-substance and mucous casts of the bronchi are readily detected. Lastly, the dish may be placed upon the stage of the microscope and its contents examined with the low power ($\frac{2}{3}$ obj.).

White Blood-corpuscles.

These are present in considerable amount in all sputa. In cases of penumonia and other pyogenic processes of the respiratory tract they may constitute the bulk of the expectorated material. They are frequently granular, fatty, or vacuolated, and may contain foreign bodies due to their phagocytic activity.

Red Blood-corpuscles.

In pulmonary hemorrhage or hemorrhage from the pharynx or post-nasal space the sputum may contain red blood-cells in greater or less quantity. Under these circumstances these cells are well preserved, while in the red sputum of pneumonia they are usually swollen and pale or crenated, and there is an admixture of leukocytes.

The color of sputum containing blood in quantity is not always red. Degenerative changes in the red blood-cells may free the hemoglobin, and chemical action may cause the sputum to assume a greenish or yellowish hue. The micro-chemical test for blood may be useful in certain cases in which the red blood-cells have been broken down and a discoloration by hemoglobin is suspected. Crystals of hematoidin are sometimes observed.

The slight discoloration of sputum by blood is usually due to its escape from the pharynx or the bronchial mucosa, and may be of little pathologic significance.

Epithelium.

Epithelial cells are always present in the sputum in large quantity. They may come from any portion of the respiratory tract, including the nasal fossæ and the mouth. Their condition is so frequently changed from the normal, by degenerations of diverse kinds, that it is not possible to identify them positively. The mononucleated, elliptic alveolar epithelium of the lungs may be present in enormous numbers in desquamative pneumonia, and also in chronic passive congestion of the lungs due to valvular or other heart lesions. In the latter case these cells contain yellow or black pigment-granules (Hertzfehlerzellen).

Upon the whole, the epithelial elements of sputum afford us little information of value.

Elastic Tissue.—Elastic fibers occurring either singly or in groups, perhaps with an alveolar arrangement, are of great diagnostic value, indicating a destructive process in the pulmonary parenchyma.

Traube has shown that in gangrene of the lung with extensive necrosis of the pulmonary substance these fibers may be absent from the sputum, and accounts for this fact by presuming that they are destroyed *in situ*. In

examining for elastic fibers von Jaksch recommends that the sputum be spread on a slide and a little caustic potash added. This clears up the sputum and the elastic fibers become visible.

If they are present in small quantity and cannot be detected by the above method, that proposed by Fenwick may be followed. He boils a quantity of sputum in an 8 or 10 per cent. solution of caustic potash, and allows the mixture to stand for twenty-four hours in a conical glass. The sediment is then removed with a pipette and examined under the microscope for elastic fibers.

On account of the fact that, despite precautionary washing of the patient's mouth prior to expectoration, elastic fibers that have been taken in as food may get into the sputum, von Jaksch contends that only when the elastic fibers show the alveolar arrangement characteristic of lung structure are they of any sure diagnostic significance.

Spirals.

Curschmann's spirals are found in the sputum of a number of conditions, though formerly they were considered to be pathognomonic of asthma. They consist usually of a central thread, around which is wound a mass of delicate fibers in a loose spiral formation. Entangled in these threads are large numbers of leukocytes, often many of them being eosinophiles, epithelial cells, and perhaps Charcot-Leyden crystals.

Czermak believes that they are formed by mucous threads that have become twisted upon their axis and thus formed them artificially. They may be present in tuberculosis, bronchitis, pneumonia, and bronchial asthma. In connection with the latter affection they are a valuable diagnostic sign, as their presence is proof that the disease

is true bronchial asthma and not of the reflex type. The spirals are usually about 1 mm. to 1.5 mm. in length, and may be seen with the unaided eye.

Fibrinous casts of the terminal bronchi may occur in the sputa of plastic bronchitis and pneumonia. In the latter condition their presence in quantity indicates a severe type of the disease. According to von Jaksch, they are sometimes several centimeters in length and finely branched. Being fibrinous in character, they resist the action of acetic acid.

Organized Tissue.—Pieces of neoplasms of the lung, sarcoma and carcinoma, as well as bits of connective tissue may be coughed up, and with the aid of the microscope their structure may be determined.

Bacteria.

The method for examining sputa for the tubercle bacillus has been already described (see p. 177).

When examination fails to reveal tubercle bacilli in sputum, *we are not warranted in making a negative diagnosis.* The bacilli may be present in numbers so small that they have escaped detection, or they may be found in a subsequent examination of sputum from the same patient, and there may arise cases of pulmonary phthisis in which it is impossible at any time to demonstrate Bacillus tuberculosis during the life of the patient.

Mittal has shown that the tubercle bacillus will multiply at 37° C. in sputum. In cases in which the organisms have not been found after repeated examinations, a specimen of the sputum should be incubated for forty-eight hours and then examined. It will by this time have liquefied, and the sediment should be used for making the preparation.

Simon recommends another method, as follows: "About 100 c.c. of sputum is boiled with double the amount of water to which from six to eight drops of a 10 per cent. solution of sodium hydrate have been added, until a homogeneous solution has been obtained, water being added from time to time to allow for evaporation. This is then centrifugated or set aside for twenty-four to forty-eight hours and examined for tubercle bacilli and elastic tissue."

Influenza.

In sputum from cases of nasal or pneumonic influenza smears stained in carbol-fuchsin (see p. 110) may show the influenza bacillus in nearly pure culture. In the early stages of the disease they usually lie free, but as the process becomes older may be found in the pus-corpuscles. They have also been demonstrated in the blood of patients suffering from influenza.

Pneumonia.

In the early stages of fibrinous pneumonia the pneumococcus is often present in the sputum in large numbers. Smears may be stained by methylene-blue and Gram's method, or animal inoculation may be resorted to (see p. 115). It must be remembered that croupous pneumonia may be caused by a variety of bacteria, and that the pneumococcus is present in 20 per cent. of healthy throats. Its presence can therefore only be considered as presumptive evidence of pneumonia.

The detection of the pneumococcus in the sputum may be of considerable value in cases of central pneumonia where the diagnosis is in doubt on account of marked physical signs. The organism and its capsule may be stained by Welch's method (see p. 114).

Actinomycosis.

When the fungus of this disease attacks the lungs, it may occur in the sputum in the characteristic granular, yellow bodies. Examination of the fresh specimen with the $\frac{2}{3}$ objective may be sufficient to identify them.

If doubt still exists, they may be stained by Gram's method. The characteristic clubs and threads will be stained a deep blue, and can be mistaken for nothing else.

Charcot-Leyden Crystals.

These crystals were at one time believed to be pathognomonic of bronchial asthma. It is now known that they occur in many other conditions, and their presence in the sputum is of little diagnostic value. They are colorless octahedral bodies, and are probably identical with the crystals of normal semen and those found in blood postmortem, and in the feces in cases of anchylostomiasis.

Hematoidin Crystals.

These may be found in sputum from cases of hemoptysis or pulmonary abscess, and indicate a previous extravasation of blood into the lung. They are in the form of columnar or needle-like clusters or rhombic crystals of a ruby red color.

"When the crystals are contained in cells, they point to a previous hemorrhage; but when free hematoidin is present in considerable quantity, the inference is that an abscess has discharged from some neighboring organ into the lung" (von Jaksch).

Crystals of Little Importance.

Cholesterin crystals, fatty crystals, tyrosin, leucin, oxalate of lime, and triple phosphate crystals have been found in the sputum, but are of little consequence from a *pathologic* standpoint.

Pulmonary Tuberculosis.

The variety of pulmonary lesions associated with tuberculosis of the lungs renders it impossible that the sputum should be in any degree constant in either its character or amount. In the acute miliary form, when the cough is slight or absent and the tubercles have not yet begun to break down, the sputum may be mucoid and tubercle bacilli absent. In the beginning stage of more chronic forms, the sputum may be muco-purulent and a few bacilli be present, while later in the disease it becomes more purulent and tubercle bacilli are present in large numbers.

The morning sputum should be invariably selected for examination.

When large abscess cavities exist, the pyogenic membrane lining them may throw off quantities of pus containing pyogenic organisms of every description, but devoid of tubercle bacilli, and the examination of pus from such cavities opened post-mortem is usually negative as regards Bacillus tuberculosis.

Acute Pneumonic Phthisis.

Here the sputum presents the aspects characteristic of ordinary fibrinous pneumonia, and is usually not examined for tubercle bacilli until after the time of the expected crisis, when the continued consolidation and high temperature sound a note of warning that arouses the suspicions of the attendant, and recourse to the microscope reveals the true nature of the malady. In such conditions the tubercle bacilli may be present in enormous numbers, though it may not be possible to demonstrate them for several days after the onset of the disease.

Lobar Pneumonia.

In the second stage—red hepatization—the sputum is

quite characteristic and of the rusty, brick color. Microscopically the following elements are distinguishable:

1. Red blood-cells—often pale and swollen or crenated
2. Leukocytes in large numbers.

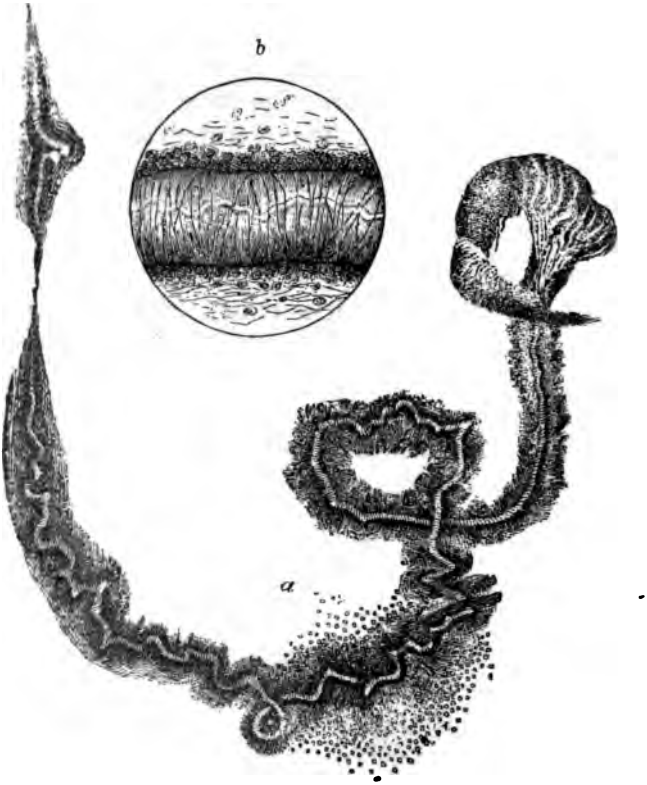

FIG. 129.—CURSCHMANN'S SPIRALS.
a, × 80 diameters. *b*, × 300 diameters.—(*Schmaus.*)

3. Epithelial cells from the alveoli and bronchial mucous membrane.

4. Bacteria of different kinds, notably the pneumococcus, and perhaps the tubercle bacillus.

Bronchial Asthma.

The sputum is viscid, white, or pink from admixture of blood, frothy, and contains Curschmann's spirals, Charcot-Leyden crystals, and usually large numbers of eosinophilic leukocytes and free eosinophile granules. A smear stained with eosin and methylene-blue will reveal the latter.

Method:

1. Sol. alcoholic eosin, ten seconds.
2. Wash.
3. Sat. aqueous methylene-blue, fifteen seconds.
4. Wash, dry, mount in balsam.

Pulmonary Abscess.

The sputum is copious, there being in some cases a primary discharge of a large quantity, and presents the general appearance of pus. Upon standing, it separates into an upper, fluid, frothy layer and a lower stratum of pus-cells. The odor may be fetid, but is not so offensive as that produced from pulmonary gangrene.

Microscopically may be seen:

1. Pus-corpuscles.
2. Epithelial cells.
3. Shreds of lung tissue.
4. Yellow elastic fibers.
5. Hematoidin crystals.
6. Cholesterin crystals.
7. Bacteria of various kinds.

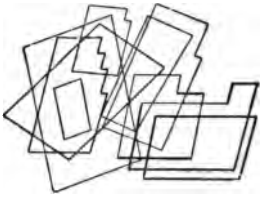

FIG. 130.—CHOLESTERIN. —(*Landois.*)

Pulmonary Gangrene.

The sputum is thin and abundant and horribly offensive, and of a brown or greenish tint. On standing, it separates into three layers, the top one thick and frothy;

the bottom, thick, opaque, greenish-brown; and a central watery, greenish-brown, semi-transparent portion.

Microscopically there are found:

1. Portions of lung tissue.
2. Elastic fibers.
3. Granular detritus.
4. Rarely crystals (hematoidin).
5. Bacteria and moulds of various kinds.

Von Jaksch denies the existence of elastic fibers in this disease, but the consensus of opinion of authorities differs with him in this particular.

Acute Bronchitis.

In the primary stage the sputum is mucoid in character, sticky, and viscid, and may show streaks of blood. Later it becomes muco-purulent and more abundant.

Microscopically will be seen:

1. Pus-cells.
2. Mucus.
3. Perhaps a few red blood-cells.
4. Bacteria of a non-specific character.

Chronic Bronchitis.

Sputum more profuse than in the acute form, and may in the case of *bronchorrhea* be quite large in amount. It is usually thin, greenish-yellow, and consists largely of pus-cells with occasional red blood and epithelial elements.

Putrid Bronchitis.

The sputum is similar to that from gangrene, but does not contain the broken-down pulmonary tissues.

Brown Induration.

Brown induration from heart disease produces a sputum which may at times be quite red in color owing to the

presence of hemoglobin derived from extravasated blood or hemorrhagic infarcts. Free red blood-cells may also be present.

Attention has already been called to the epithelial cells of the sputum of this affection, the so-called "Hertzfehlerzellen" (heart-failure cells). They consist of desquamated alveolar epithelium which contains granules of yellowish pigment (hematoidin).

Pneumonoconiosis.

The sputum of the various mechanical pigmentations of the lungs shows epithelial cells containing granules of the special kind of pigment present—coal, iron, stone, etc

STOMACH CONTENTS.

The normal gastric juice is a clear or slightly turbid fluid, acid in reaction, and containing a few squamous cells, which have desquamated from the mouth or esophagus, and bacteria of a variety of kinds. Some of the latter are known to take part in the physiologic digestion of food through the action they exert upon the carbohydrates and proteids.

MICROSCOPIC EXAMINATION OF VOMIT.

A large drop of the vomit is placed upon a glass slip, covered with a cover-glass, and examined unstained, first with the low and then with the high power. A great variety of substances may be seen, including partly

digested food, muscle fibers, connective-tissue, yellow elastic fibers, fat-globules, vegetable cells and fibers, mucus, mucus-corpuscles, sarcinæ, and bacteria of other forms, etc.

In pathologic conditions there may be blood, in cases of gastric ulcer or carcinoma; or pus, denoting suppurative conditions of the mucosa, or, if it is present in large quantity, the rupture into the stomach or esophagus of a neighboring abscess.

In carcinoma of the stomach there may be found portions of the malignant growth. These must be differentiated from shreds of the mucosa.

The peculiar arrangement of the carcinoma cells within alveoli of connective tissue, and the fact that the character of the cells differs from that of the cells constituting the normal mucosa, are usually sufficient to enable one to come to the correct conclusion. On the other hand, the old idea that the cancer cell possesses features peculiar to itself, so that a diagnosis may be made from *individual detached cells, is entirely erroneous.*

Blood in vomited material, if in small quantity, may be difficult of demonstration, from the fact of the probable destruction of the corpuscles. In such a case the microchemical test for blood already mentioned should be tried with some filtrate from the vomit which has been dried upon the glass slip.

FECES.

The feces consist of the remains of undigested food, secretions of the intestinal tract, desquamated epithelium from the gastro-intestinal mucosa, and bacteria.

Microscopic Elements.

Upon minute examination the bulk of the feces will be found to be composed of remnants of food, the character of the passage therefore depending much upon the previous diet. Usually there are seen striated muscle, often much altered in form and devoid of striations, connective and elastic tissue, starch granules, vegetable cells, spirals, and fat-globules. Needle-shaped crystals occurring singly or in bundles may be seen. These are derived from the fatty acids.

Triple phosphate crystals are not infrequent, as are other crystalline forms, but these are of no diagnostic importance. A large amount of granular material that cannot be identified remains; this is called detritus.

Formed Elements.

These are derived from the intestinal tract.

Red Blood-corpuscles.

It is seldom possible to find red blood-corpuscles in feces unless from a hemorrhage very low down in the intestine or rectum. Usually even in severe hemorrhage the red cells are destroyed by the intestinal juices, so that while the feces may be intensely colored by the liberated hemoglobin, one may search in vain for red blood-corpuscles. The microchemical test performed with a drop of the feces will show the characteristic hemoglobin crystals if the color is due to free blood.

Leukocytes.

In normal stools these elements are uncommon, but in catarrhal or inflammatory diseases of the intestines they may occur in large numbers. If a quantity of pus is sud-

denly expelled per rectum, it may be due to the discharge of an abscess into the intestinal tract.

Epithelium.

In health few epithelial elements are found in the feces, though a variety of forms may be present. In desquamative processes in the intestinal tract they may occur in large numbers. It is only when they are present in quantity that they are of any clinical importance.

Bacteria.

Enormous numbers of bacteria occur constantly in the feces. They are largely derived from the food ingested and from the saliva. Many of them are saprophytes and harmless, while others may, under conditions favorable to them, produce serious lesions.

The more important organisms that have been isolated from the feces are Bacillus typhosus, the colon bacillus, Bacillus lactis aerogenes, Bacillus proteus vulgaris, the spirillum of Asiatic cholera, and the tubercle bacillus; these have already been considered.

Trichina spiralis.—Trichinosis is contracted by man by eating meat infected with the parasite, which is common in hogs (measly pork). The male worm is about 1.5 mm. long, the female 3 mm. long. After fertilization in the intestinal canal the parasites penetrate the mucosa, and the adult forms eventually become encysted in the skeletal muscles, the psoas and the muscles of the thigh being chiefly affected. In sections of muscles viewed with the low power (⅔ obj.) the parasites are seen coiled within the oval cysts. A rich capillary plexus is formed about the cysts, as is well shown in Fig. 131.

Pieces of suspected meat may be pressed thin between two glass slips and examined without staining. Cats are

frequently affected by this parasite. Rarely it is found in human feces in infected cases, or where infected meat has been ingested.

The eosinophilia occurring in trichinosis is mentioned in the section on blood.

Amœba coli.—In the amebic form of dysentery and in liver abscesses Lösch in 1875 discovered bodies possessing the power of ameboid movement, but these cells were not recognized by him as being in any way the cause of the disease.

It remained for Kartulis to point out that tropic dysentery and liver abscesses following these cases were due to Amœba coli, and his observations have been repeatedly confirmed by other observers.

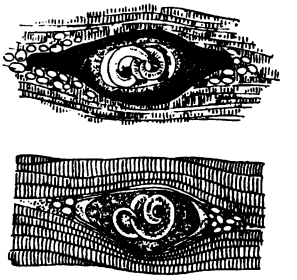

FIG. 131.—TRICHINA SPIRALIS.—(*Reeves.*)

At rest, the ameba is a cell-like circular or oval body of 10 μ to 20 μ in diameter. The ameba may present great irregularity of form, and, in addition to a nucleus, contain granulations and vacuoles.

In fresh feces from a case of amebic dysentery, on the warm stage they may show active amebic movement, projecting pseudopodia, and streaming of the granular protoplasm. They may also be demonstrated in stained sections of colon or liver infected with them, and in the pus of liver abscess, of which they are the causative agent.

Hydatid Cysts.

The microscopic appearances of the fluid from a hydatid cyst are very characteristic. The fluid should be allowed to stand in a conical glass, and the sediment, examined

with the low power (⅔ obj.), should show the hooklets and scolices of the ecchinococcus or portions of membrane which on their inner surface are granular and distinctly striated.

The hooklets look like minute cat's claws, while the scolices are composed of a double circular row of hooklets and four suctorial discs attached by a constricted neck to a short pouch-like body.

The adult form of the parasite is found in the intestinal canal of dogs as *Tænia echinococcus*.

The cysts may form in various portions of the body or break into the respiratory or genito-urinary tract, in which case the hooklets or membrane may be found in the sputum or urine. They may also occur in feces.

Ovarian Cysts.

The contents of ovarian cysts vary greatly according to the nature of the cyst. Usually the formed elements are very numerous. In dermoid cysts there may be found in the aspirated fluid numerous squamous epithelial cells, hairs, and crystals—fatty, hematoidin, or cholesterin. The latter may be recognized as clear plates, often superimposed, square or rhomboidal, with a square notch in one corner.

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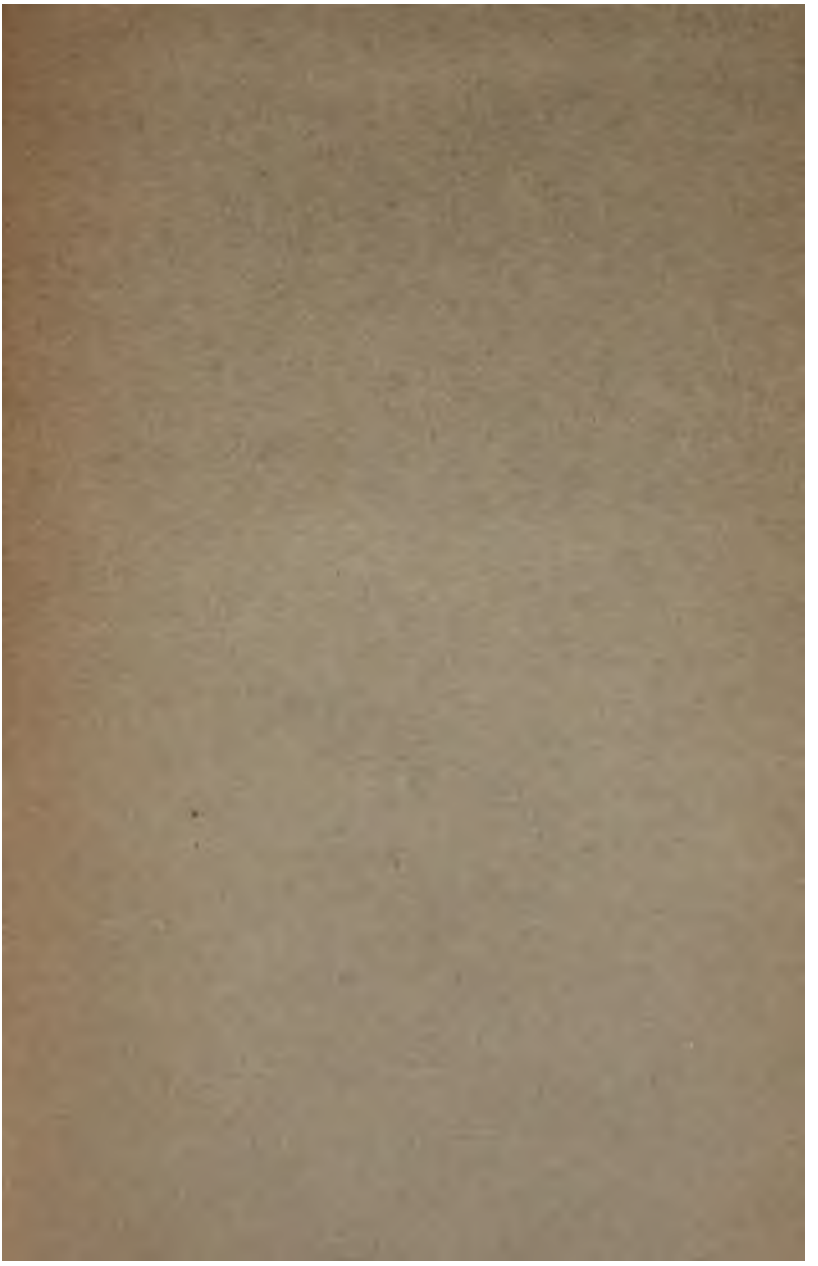

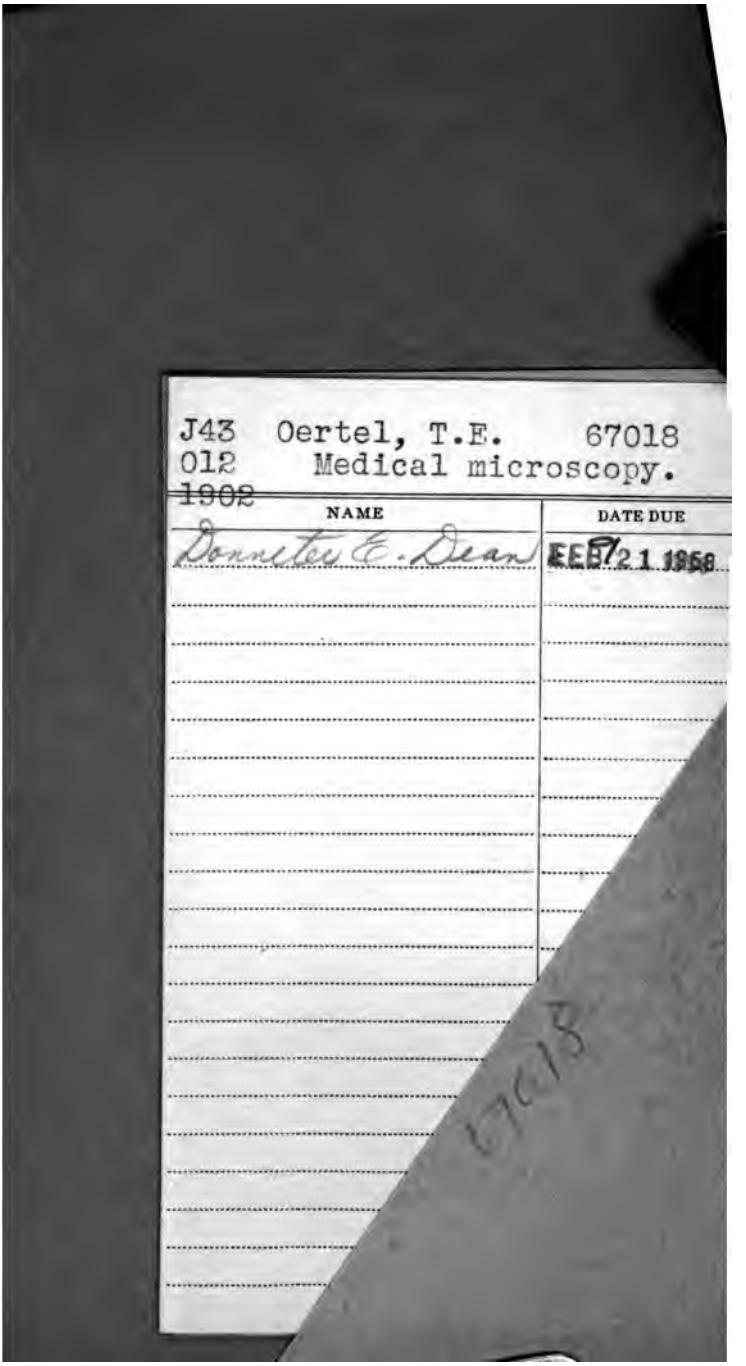
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